Vol. 38, No. 2 June 1951

THE ANNALS OF APPLIED BIOLOGY

EDITED FOR THE ASSOCIATION OF APPLIED BIOLOGISTS

BY

R. W. MARSH

AND

I. THOMAS

WITH THE ASSISTANCE OF

F. J. ANSCOMBE J. T. MARTIN
F. C. BAWDEN D. J. WATSON

CAMBRIDGE UNIVERSITY PRESS
LONDON: BENTLEY HOUSE, N.W.1
NEW YORK: 51 MADISON AVENUE

The Association of Applied Biologists

G. FOX WILSON

President, 1949–50

OBITUARY

MR G. FOX WILSON

George Fox Wilson joined the Association in 1920, was on the Council 1927–9, served as Zoological Secretary 1933–4, as General and Zoological Secretary 1935–6 and again as Zoological Secretary 1937–8, and was President in 1949 and 1950.

Fox Wilson's merry smile, his cheerful conversation, his personal charm, the reality of his friendship, his clarity of exposition whether it be to student or colleague, in council or in public, the soundness of his knowledge, his unswerving loyalty to the societies he served and the complete devotion to the task in hand will not soon be forgotten.

Born on 26 January 1896, Fox Wilson was educated at King Edward VI School, Grantham, and entered the gardens of the Royal Horticultural Society at Wisley as a student in 1911. Here, after 2 years, the Worshipful Company of Gardeners awarded him a scholarship and he worked as assistant to Prof. Maxwell Lefroy, who was then entomologist at Wisley, until 1915. In this year Fox Wilson joined the 1st City of London Sanitary Company, and was stationed for 3 years in Egypt and Syria, where his principal work was on anti-malaria measures and control of the fly pests. On his return in 1919 he was appointed entomologist to the Royal Horticultural Society which he served until his death on 9 January 1951.

His life was devoted to entomology as it concerns horticulture, and his long tenure of the post of entomologist to the Royal Horticultural Society provided the necessary opportunities. Quietly and without any fuss he studied the pests of ornamental plants and the means of overcoming them. He taught the students at Wisley and Kew. He advised gardeners, commercial growers and official entomologists. Throughout the years a steady stream of publications appeared explaining the results of his investigations on such diverse aspects of horticultural entomology as pollination in orchards, the prevention of insect attacks in gardens, eelworm pests of phlox and other plants, insects associated with seeds, rhododendron white fly, pests of vegetables, DDT, Gammexane, HETP and recent developments in glasshouse fumigation. He took a leading part in the dissemination of the white fly parasite and the mapping of the spread of non-indigenous pests. Much of his knowledge is incorporated in his *Pests of Ornamental Garden Plants* (Bulletin no. 97, 1937, Ministry of Agriculture and Fisheries), the second edition of which is *Pests of Flowers and Shrubs* (Bulletin 97, 1950), and in *The Detection and Control of Garden Pests* (1st edition 1947, 2nd edition 1949, Crosby Lockwood and Son Ltd.).

Imperceptibly, without the recognition he merited, Fox Wilson had become one of the outstanding entomologists of the country and as an horticultural entomologist

he had no peer. Early in his career he gained the National Diploma in Horticulture and had been elected a Freeman of the City of London. In 1920 he joined the (Royal) Entomological Society of London, served on its Council (1935–7, 1940–2 and 1946–8) and was one of its Vice-Presidents (1940, 1941 and 1947–8).

Who will not remember our President, standing on the steps at Wisley, hands in pockets, slightly stooping, smiling with happiness and pride, when he welcomed, in his capacity as senior member of the staff of the Wisley laboratories and representative of the Royal Horticultural Society, the Association on its recent visit?

Or who will forget him, standing before the brightly caparisoned and most dignified Lord Provost, Magistrates and Councillors of Edinburgh, replying to the address of welcome at first hesitatingly, then as a most distinguished President reducing that august company to unfeigned laughter and outright appreciation?

But Fox Wilson was a tired man. Early in 1950 his heart was showing signs of fatigue. Yet work and responsibility did not decrease, and though willing he could not go on indefinitely. Early in December he suffered a cerebral thrombosis from which he never recovered.

In our happiness and gratitude for having known such a friend, teacher, adviser and colleague, we must remember his widow in her bereavement and their two young sons.

H. F. Barnes

LIST OF PUBLICATIONS, 1913–1951

Insects attacking apple, pear, plum and cherry. *Four essays written by students at Wisley, 1913*, pp. 44–72.

Injurious insects, and how to fight them. *Gdnrs' Mag.* 31 Jan. and 7 Feb. 1914, pp. 95–6 and 117–18.

Bird life in the Suez Canal area. *J. R. hort. Soc. Gdns Cl.*, no. 12, 1919, pp. 7–9.
Spraying and spraying machinery. *Fruit Fr. J., Lond.*, 5 and 12 July, 1919, pp. 7 and 35–6.

Insect pests in the R.H.S. Gardens, 1919–20. *J. R. hort. Soc. Gdns Cl.*, no. 13, 1920, pp. 7–12.

Wild plants as hosts of insect pests at Wisley. *J. R. hort. Soc. Gdns Cl.*, no. 14, 1921, pp. 5–8.
Spraying and spraying machinery, pp. 76–80, in *Fruit Farming* by C. H. Hooper. London: Lockwood Press. 1921.

(With A. N. Rawes): Pollination in orchards (VI). Pollen-carrying agents. *J. R. hort. Soc.* **47**, 1922, 15–17.
Apple blossom weevil. *Fruitgrower*, 31 Aug. 1922, p. 384.

Otiorrhynchus rugifrons and *O. sulcatus* as pests of Alpine plants. *Ent. mon. Mag.* **59**, 1923, 38–9.
Melandrya caraboides larva attacking plum trees. *Ent. mon. Mag.* **59**, 1923, 199–200.
Otiorrhynchus picipes F. and *Strophosomus coryli* F. attacking rhododendrons, and *Xyleborus dispar* F. destroying red-currant bushes. *Ent. mon. Mag.* **59**, 1923, 200.
The woolly aphis of the Auricula. *Gdnrs' Chron.* **74**, 1923, 312.
Hosts (wild) for fruit pests. *Fruitgrower*, 22 Nov. 1923, p. 897.

Thereva plebeia L. larvae attacking cabbages and potatoes. *Ent. mon. Mag.* **60**, 1924, 16–17.
Thereva plebeia as a pest of economic importance. *J. R. hort. Soc.* **49**, 1924, 197–202.
The eelworm disease of phloxes. *J. R. hort. Soc.* **49**, 1924, 203–10.
Report upon trials of grease-banding materials 1923–24. *J. R. hort. Soc.* **49**, 1924, 237–41.
The eucalyptus Psylla, *Eurhinocola eucalypti* (Maskell). *Gdnrs' Chron.* **76**, 1924, 425.

Mosquito destruction. Essay, College of Pestology. 1 Jan. 1925.
The egg of the tarnished plant bug, *Lygus pratensis* Linn. *Ent. mon. Mag.* **71**, 1925, 19–20.
Insect pests of rhododendron. *J. R. hort. Soc.* **50**, 1925, 46–54.
Mosquito control in gardens. *The Garden*, 27 June 1925, pp. 362–3.
Entomological congress at Zurich. *Gdnrs' Chron.* **78**, 1925, 133–4.
Missing butterflies. *Gdnrs' Chron.* **78**, 1925, 375.
The mining habit of *Otiorrhynchus rugifrons* larvae. *Ent. mon. Mag.* **61**, 1925, 273–6.

Pollination in orchards (VII). Insect visitors to fruit blossoms. *J. R. hort. Soc.* **51**, 1926, 225–51.
Tetrachlorethane as a glasshouse fumigant. *Gdnrs' Chron.* **79**, 1926, 138.
A weevil pest in Alpine plants. *The Garden*, 22 May 1926, p. 283.
Insect visitors to sap-exudations of trees. *Trans. ent. Soc. Lond.* **74**, 243–53.
Is there an insect menace? (Symposium). *Mod. Sci.* **7**, 1926, 466.

Narcissus eelworm (in Daffodil Conference 1926). *J. R. hort. Soc.* **52**, 1927, 62–5.
A short history of spraying machinery in England. *Gdnrs' Chron.* **81**, 1927, 13–14.
Insect pests of garden plants. *The Garden*, 23 Apr. 1927, pp. 267–8.
Enemies of the rose. *The Garden*, 30 Apr. 1927, pp. 282–3.
A menace to toads. *Gdnrs' Chron.* **82**, 1927, 93.
The prevention of insect attacks in gardens. *J. R. hort. Soc.* **52**, 1927, 235–45.
Grease-banding fruit trees. *Country Life*, 1 Oct. 1927, pp. 485–6.

Plant lice (Aphides) and their control. *Gdnrs' Yearb. 1928*, 1928, pp. 249–53.
Some pests of water lilies. *J. R. hort. Soc.* **53**, 1928, 81–91.
Two lesser known pests of fruit trees. *Gdnrs' Chron.* **83**, 1928, 416–18.
Nicotine as an insecticide. *The Chemist & Druggist*, 31 Mar. 1928, pp. 423–4.
The grease-banding of fruit trees. *Gdnrs' Chron.* **84**, 1928, 255.
Congress of Entomology. *Gdnrs' Chron.* **84**, 1928, 315–17.

Effect of weather on gooseberry sawfly and bees when pollinating fruit trees. *Conf. Empire Meteorologists; Agric. Sect., II Papers and Discussions*, 1929, pp. 161–3.
The rhododendron white fly. *J. R. hort. Soc.* **54**, 1929, 214–17.
Pollination of hardy fruits: insect visitors to fruit blossoms. *Ann. appl. Biol.* **16**, 1929, 602–29.
A Dipterous pest of Berberis and Cotoneaster species. *Gdnrs' Chron.* **86**, 1929, 409–10.

Further investigations on the eelworm disease of phloxes. *J. R. hort. Soc.* **55**, 1930, 88–100.
Insect pests of garden plants: symptoms of attack. *Country Life*, 2 Aug. 1930, pp. 163–4.

Mealy bugs and their control. *Gdnrs' Yearb. 1931*, 1931, pp. 89–93.
Insect associated with the seeds of garden plants. *J. R. hort. Soc.* **56**, 1931, 31–47.
Biological control of the greenhouse white fly. *Gdnrs' Chron.* **89**, 1931, 15–17.
Insect pests of hardy rhododendrons: with notes on some uncommon damage. *Gdnrs' Chron.* **89**, 1931, 244–5.

Garden pests: their detection and control. *Gdnrs' Chron.* **91**, 1932, 186–8, 242–3, 278–9, 294–5, 349–50, 366–7, 464–5, 478–9; **92**, 1932, 12, 26–7, 144–5, 161, 217, 270–1; **93**, 1933, 28, 175, 230, 278–9, 421; **94**, 1933, 12–13, 147, 204–5, 294, 330–1, 436–7; **95**, 1934, 14–15; **96**, 1934, 59–60; **97**, 1935, 10–12, 309, 399–400; **98**, 1935, 11–12, 180–1, 354–5; **100**, 1936, 182, 218, 273, 288–9, 412, 451.

On the alleged larvicidal properties of *Chara* species. *Proc. Linn. Soc., Lond.*, 1932, pt. 4, pp. 131–2.

Pollination in orchards (VIII). Insect visitors to fruit blossom. *J. R. hort. Soc.* **58**, 1933, 125–38.
Insect pests of hardy rhododendrons: their detection and control. *New Flora and Silva*, **6**, 1933, 36–44.
Symptomatic detection of plant pests: its importance in the training of agricultural, horticultural, and forestry students. *Proc. 5th Congr. int. Ent. Paris 1932*, 1933, pp. 519–28.

The rock ape. *J. R. hort. Soc. Gdns Club*, no. 26, 1934, pp. 8–9.
The stem eelworm. *Leaflet R. hort. Soc.* 1934, 4 pp.
Insect pests of garden irises: their detection and control. *Irish Yearb. 1934*, 1934, pp. 77–86.
Woolly aphis and nasturtium (*Tropaeolum*): a fallacy. *Gdnrs' Chron.* **96**, 1934, 391.
How to deal with fruit pests. *The Listener*, 19 Dec. 1934, p. 1039.

Additional hosts of *Anomoea antica* Wied. and *Spilographa alternata* Fall. *Ent. mon. Mag.* **71**, 1935, 58–60.
Phylloxera on vines. *J. R. hort. Soc.* **60**, 1935, lx-lxi.
The rhododendron white fly. *J. R. hort. Soc.* **60**, 1935, 264–71.
Phylloxera on vines, a new British record. *Proc. R. ent. Soc. Lond.* **10**, 1935, 25–8.
Fruit pests: their effect and detection. *J. R. hort. Soc.* **60**, 1935, 536–44.

Insects visiting *Nymphaea stellata*. *J. R. hort. Soc.* **61**, 1936, xxxix-xl.
Pollination of soft fruits. *Bull. Minist. Agric. Lond., Fruit Production*, no. 4, 1936, pp. 70–1.

Insects captured in the flowers of *Nymphaea capensis* Thumb. *Proc. R. ent. Soc. Lond.* (A), **12**, 1937, 31–9.
Some common pests of the flower garden. I. *Country Life*, **81**, 1937, 364–6.
Some common pests of the flower garden. II. *Country Life*, **81**, 1937, 386–7.
A new pest of rhododendrons. *Yearb. Rhododendron Ass. 1937*, 1937, pp. 56–7.
A leaf-hopper (Jassid) on rhododendron. *J. R. hort. Soc.* **62**, 1937, 210–13.
Gryllotalpa gryllotalpa Linn. in a Hampshire garden. *Ent. mon. Mag.* **73**, 1937, 162.
Pests of ornamental garden-plants. *Bull. Minist. Agric. Lond.*, no. 97, 1937, 128 pp., 36 pls.
The root-knot eelworm, *Heterodera marioni* (Cornu) Goodey, and its relation to plants growing outdoors in the British Isles and in certain European countries. *J. R. hort. Soc.* **62**, 1937, 336–46.
Seasonal operations against garden pests. *Country Life*, 1937, pp. cxlvi–cxlvii.

Pests of commercial ornamental plants. *Sci. Hort.* **6**, 1938, 102–16 and *Univ. Reading. Fac. Hort.* 1938, Bull. L, pp. 102–16.
Villains of the piece. *Good Gardening*, **4**, 1938, 22, 23 and 57.
Insect friends. *Good Gardening*, **4**, 1938, 41–2.
The tarnished plant bug or bishop fly, *Lygus pratensis* L.—Précis of present knowledge. *J. R. hort. Soc.* **63**, 1938, 392–5.
Pests and diseases of bearded irises. *Gdnrs' Chron.* **104**, 1938, 114–16.
Bishop fly and chrysanthemums. *Gdnrs' Chron.* **104**, 1938, 130–1, 147.
The glasshouse leaf-hopper, *Erythroneura pallidifrons* Edw. *J. R. hort. Soc.* **63**, 1938, 481–4.

Insect pests of rhododendrons: their distribution in Britain. *Proc. R. ent. Soc. Lond.* (A), **14**, 1939, 1–5.
Ware these flower pests. *Popular Gardening*, 13 May 1939, pp. 136.
Destructive pests of early summer. *Good Gardening*, **5**, 1939, 22–3, 48–9.

The control of insect pests and diseases: spraying and dusting. *Gardening Illustrated*, **61**, 1939, 409–10.
Amaryllis pests. *J. R. hort. Soc.* **64**, 1939, 318–26.
Plant galls. *The Country-Side Diary 1940*, 1939, Note for 45th week, 6–7 Nov.
Insect pests of the genus Rhododendron. *Proc. VII Congr. int. Ent. Berlin 1938*, 1939, (1944: 1947), **4**, 2296–2323.

Spring time is spraying time. *Gardening Illustrated*, 9 Mar. 1940, pp. 125–6.
The sexual ratio of the common earwig, *Forficula auricularia* L. (Dermapt.) as observed in trap bands. *Proc. R. ent. Soc. Lond.* (A), **15**, 1940, 17–20.
Phenological observations on the brimstone butterfly, *Gonepteryx rhamni* (Linn.) (Lepid.). *Proc. R. ent. Soc. Lond.* (A), **15**, 1940, 13–16.
The cabbage white butterfly, *Pieris brassicae* Linn. *J. R. hort. Soc.* **65**, 1940, 278–81.
Some seasonal pests of garden vegetables and their control. *J. R. hort. Soc.* **65**, 1940, 407–19.

Total warfare against garden pests. *Gardening Illustrated*, 25 Jan. 1941, pp. 44–5.
Some delphinium pests. *Delphinium Yearb. 1941*, 1941, pp. 7–9.
Hygiene in the kitchen garden. *Gardening Illustrated*, 22 Mar. 1941, pp. 142–3.
Hygiene in the fruit garden. *Gardening Illustrated*, 29 Mar. 1941, pp. 154–5.
The gardener's spring offensive. *Country Life*, 12 Apr. 1941, pp. xvii-xviii.

Hygiene in the war-time fruit garden: winter operations. *J. R. hort. Soc.* **67**, 1942, 28–30.
The lily beetle, *Crioceris lilii* Scop. *J. R. hort. Soc.*, **67**, 1942, 165–8.
Aphides, with special reference to their control. *J. R. hort. Soc.* **67**, 1942, 199–205.
On keeping fruit trees clean. Rewritten, 6th ed. 1942.
The invasion of houses by earwigs and ants. *Ann. appl. Biol.* **29**, 1942, 316–21.
The harvesting of alder buckthorn (*Rhamnus frangula*) and the effect on the insect fauna. *Proc. E. ent. Soc. Lond.* (A), **17**, 1942, 112.
The bean aphis and its control. *J. R. hort. Soc.* **68**, 1943, 49–52.

A flea-beetle trap. *J. R. hort. Soc.* **68**, 1943, 106–7.
Potato tuber injury due to soil pests. *J. R. hort. Soc.* **68**, 1943, 206–14.
The daffodil fly. *J. R. hort. Soc.* **68**, 1943, 236–9.
A note on the control of the onion fly, *Delia antiqua*. *J. R. hort. Soc.* **68**, 1943, 276–7.
Carrot aphides. *Amateur Gardening*, 18 May, 1943.
The chrysanthemum eelworm. Leaflet Nat. Chrysanthemum Soc., 1943, 6 pp.
Keeled slugs. *Amateur Gardening*, 7 Dec. 1943.
Woolly aphis in winter. *Amateur Gardening*, 14 Dec. 1943.
The lily beetle, *Crioceris lilii* Scopoli: its distribution in Britain. *Proc. R. ent. Soc. Lond.* (A), **18**, 85–6.
The stem and bulb eelworm, *Anguillulina dipsaci* (Kühn, 1858): the importance of collating evidence on the behaviour of biologic strains. *Ann. appl. Biol.* **30**, 1943, 364–70.

Raspberry cane midge. *J. R. hort. Soc.* **69**, 1944, 79–83.
Earwigs in the garden. *Amateur Gardening*, 21 Mar. 1944.
(With D. E. Green): A simple calendar of control measures against vegetable pests and diseases in gardens and allotments. *J. R. hort. Soc.* **69**, 1944, 104–11.
Watch vegetable flies now. *Amateur Gardening*, 4 Apr. 1944, p. 12.
Ants in the garden. *Amateur Gardening*, 27 June 1944, p. 8.
The yew scale. *J. R. hort. Soc.* **69**, 1944, 244–8.
The control of mushroom flies. *Amateur Gardening*, 3 Oct. 1944, p. 9.
More about winter washes. *Amateur Gardening*, 13 Feb. 1944, p. 12.

The carrot fly and its control in gardens. *J. R. hort. Soc.* **70**, 1945, 84–9.
The leopard moth. *J. R. hort. Soc.* **70**, 1945, 148–50.

Small-scale carrot fly control. *Agriculture*, **52**, 1945, 219–23.
The rosy rustic moth. *Amateur Gardening*, 7 Aug. 1945, p. 8.
Insect pests of *Cotoneaster horizontalis*. *J. R. hort. Soc.* **70**, 1945, 271–5.
Investigations on the control of carrot fly (*Psila rosae* F.) in gardens. *Ann. appl. Biol.* **32**, 1945, 265–76.

DDT: investigations on its effect upon some horticultural pests. *J. R. hort. Soc.* **71**, 1946, 6–13.
The chrysanthemum eelworm. *Nat. Chrysanthemum Soc. Yearb. 1945*, 1946, pp. 17–33.
Post-blossom fruit spraying. *Amateur Gardening*, 7 May 1946, p. 11.
Bulb eelworm problem. *Amateur Gardening*, 21 May 1946, p. 14.
Some pests of deciduous ornamental trees and shrubs. *J. R. hort. Soc.* **71**, 1946, 193–202.
Factors affecting populations of social wasps, *Vespula* species, in England (Hymenoptera). *Proc. R. ent. Soc. Lond.* (A), **21**, 1946, 17–27.
The eelworm disease of phloxes. *Bull. Misc. Information, Hortic. Trades Assoc.* no. 2, 1946, pp. 3–6.
Recent developments in garden pest control. *J. R. hort. Soc.* **71**, 1946, 334–43.
The chrysanthemum eelworm. Leaflet Nat. Chrysanthemum Soc., 1946, 17 pp.

The leaf-rolling rose sawfly, *Blennocampa pusilla* Klug. *J. R. hort. Soc.* **72**, 1947, 155–8.
The chrysanthemum eelworm and its control. *J. R. hort. Soc.* **72**, 1947, 364–9.
The specific uses of DDT and Gammexane in horticulture, 1947, pp. 77–88. Revision course in horticulture, Midland Agric. Coll., 3–7 Sept. 1946. Private Circulation.
(With D. E. Green): *Clean fruit—apple and pear*. R. hort. Soc. Leaflet, 1947, 16 pp.
The detection and control of garden pests. 1947, 194 pp., 54 pls. London: Crosby Lockwood and Son Ltd.
Grease-banding. *Popular Gardening*, 20 Sept. 1947, p. 268.
Warm-water sterilization. *Popular Gardening*, 27 Sept. 1947, p. 278.
Soil pests. *Popular Gardening*, 11 Oct. 1947, p. 298–9.
Greenhouse hygiene. *Popular Gardening*, 25 Oct. 1947, p. 318.
Winter hygiene in the flower garden. *Popular Gardening*, 8 Nov. 1947, p. 336.
Hygiene in the fruit garden. *Popular Gardening*, 22 Nov. 1947, p. 354.
Winter hygiene in the vegetable garden. *Popular Gardening*, 6 Dec. 1947, pp. 369, 372.
The large narcissus or daffodil fly. *The Daffodil and Tulip Yearb. 1947*, 1947, pp. 60–2.
Winter spraying: why it is essential. *Popular Gardening*, 27 Dec. 1947, p. 396.

Winter spraying: egg-killing washes for fruit pests. *Popular Gardening*, 1 Jan. 1948, p. 400.
Spraying equipment for amateur fruit growers. *Popular Gardening*, 10 Jan. 1948, pp. 410, 416.
Two injurious aphid pests of conifers. *J. R. hort. Soc.* **73**, 1948, 73–8.
Fruit pests. *Popular Gardening*, 17 April 1948, p. 64.
Vegetable pests. *Popular Gardening*, 1 May 1948, pp. 82, 84.
Flower pests. *Popular Gardening*, 22 May and 5 June 1948, pp. 111, 126, 128.
The rhododendron white fly. Minist. Agric. Lond. Leaflet for Inspectors.
The cabbage butterfly. *Amateur Gardening*, 13 July 1948, p. 13.
Cabbage aphides. *Amateur Gardening*, 20 July 1948, p. 13.
The bulb and stem eelworm in relation to garden plants. *J. R. hort. Soc.* **73**, 1948, 335–45.

The small narcissus fly in relation to lily bulbs. *The Lily Yearb. 1948*, no. 12, 1949, pp. 71–2.
HETP—a nicotine substitute. *J. R. hort. Soc.* **74**, 1949, 116–21.
Control of rose pests. Recent developments. *The Rose Annual 1949*, 1949, pp. 52–6.
The specific uses of DDT and Gammexane in horticulture. *Sci. Hort.* **9**, 1949, 67–78.
Maggot-free raspberries. *Amateur Gardening*, 31 May 1949, p. 19.
The detection and control of garden pests, 2nd ed. 1949, 194 pp., 54 pls. London: Crosby Lockwood and Son Ltd.

Recent developments in glasshouse fumigation. *J. R. hort. Soc.* **74**, 1949, 444–51.
(With J. Ramsbottom): *The Enemies of the Rose.* Nat. Rose Soc., 1949.

The broad mite. *J. R. hort. Soc.* **75**, 1950, 69–74.
Pests of ornamental plants. *The Times Gardening Supplement*, March 1950, p. 16.
Friendly insects. *The Rose Annual 1950*, 1950, pp. 13–19.
Chrysanthemum pests. *Nat. Chrysanthemum Soc. Yearb. 1949*, 1950, pp. 3–16.
Control of bulb eelworm. *Amateur Gardening*, 20 June 1950, p. 12.
Bark beetles. *J. Park Administration*, **15**, 1950, 63–4.
Cutworms. *Amateur Gardening*, 15 Aug. 1950, p. 20.
Glasshouse fumigation. *Gdnrs' Chron.* **128**, 1950, 190–1.
Developments in glasshouse fumigation in Britain. *Proc. VIII Congr. int. Ent., Stockh. 1948*, 1950, pp. 892–909.
Pests of flowers and shrubs. *Bull. Minist. Agric. Lond.*, no. 97, 2nd ed. (new title), 1950, 105 pp., 40 pls.

Modern trends in insecticidal control. *J. R. hort. Soc. Gdns Cl.*, no. 42 (1949), 1951, pp. 17–18.
The clay coloured weevil. *The Rose Annual 1951*, 1951, pp. 144–5.
Systemic insecticides. *J. R. hort. Soc.* **76**, 1951, 165–8.

Other Obituary Notices have appeared as follows:

The Times, 11, 16 and 17 January, 1951.
The Grower, **35**, 1951, 61 (13 Jan.).
Gardeners' Chronicle, **129**, 1951, 24 (20 Jan.).
Nature, Lond., **167**, 1951, 259 (17 Feb.).
Ent. mon. Mag. **87**, 1951, 94 (March).
J. R. hort. Soc. **76**, 1951, 117–18 (April) (with a portrait as a young man).
The Rose Annual 1951, 1951, p. 145 (April).

STUDIES ON SYSTEMIC FUNGICIDES. I. FUNGICIDAL PROPERTIES OF THE ARYLOXYALKYLCARBOXYLIC ACIDS

By S. H. CROWDY
Long Ashton Research Station (University of Bristol)

and R. L. WAIN
Wye College (University of London)

(With 2 Text-figures)

A method is described for assessing the systemic activity of compounds in checking the infection of broad bean (*Vicia faba*) by the fungi *Botrytis cinerea* or *B. fabae*. Treatment consisted in allowing the roots of young seedlings to stand in a solution containing 10 p.p.m. of the chemical for 2–3 weeks. The plants, together with controls, were then inoculated and when symptoms had had time to develop, the degree of chocolate spot infection was assessed. Several methods of disease assessment were examined and are critically discussed.

Certain phenoxyalkylcarboxylic acids tested by this method consistently gave a reduction in the mean size of fungus lesions on the bean leaves, clearly indicating systemic fungicidal action. The most promising substances were 2:4:6-trichlorophenoxyacetic, pentachlorophenoxyacetic and pentachlorophenoxy*iso*butyric acids. Further experiments with these compounds involving soil treatment, stem injection and spray application are described, and in most cases systemic fungicidal activity was clearly demonstrated. Certain compounds caused visible damage to the plants or resulted in a reduction in growth.

The results presented indicate that phenoxy acids can be fairly readily translocated in bean plants and that they tend to accumulate in actively growing tissues. It is considered unlikely, however, that they persist for long periods in plant tissue.

In the soil, the compounds appeared to remain effective for a considerable time, particularly the less soluble pentachloro acids, suggesting that soil application might provide a safe and useful method of treatment.

Introduction

Fungicides and fungicidal activity have been the subject of a considerable amount of study, but in the main the search has been for chemicals which are effective in protecting plants against infection. Although fungicides of this type have proved highly successful in practice, only those parts of the plant which are actually covered by the fungicides are protected and all fresh growth is liable to attack. Further, protectant fungicides are of no value in treating diseases which are already established in the surface tissues or vascular diseases. These disadvantages might, to a large extent, be overcome by the use of compounds which could be taken up by the plant and act directly or indirectly on the pathogen within the tissues; the treatment of diseases in this way is referred to as chemotherapy. The early work on plant chemo-

therapy (for review see Horsfall, 1945), was mainly directed towards the treatment of vascular diseases. The symptoms associated with such a disease may result either from the development of the pathogen itself or from toxins which it excretes into the transpiration stream. It follows, therefore, that these symptoms might be reduced either by checking the development of the pathogen or by antidoting the toxin it produces. Some success in both these directions has been reported; Howard (1941) was the first to record success in antidoting toxins produced by *Phytophthora cactorum* in the bleeding canker disease of maples, while Zentmyer (1942) demonstrated that injection of certain chemicals prior to inoculation with *Ceratostomella ulmi* would reduce the rate of infection with Dutch elm disease, thus indicating a direct effect on the fungus.

In some respects the treatment of leaf diseases presents a simpler picture than the treatment of vascular diseases since the activity of the fungus is often restricted to the tissues immediately adjacent to the site of the original infection. For successful results, any compound used for such treatment must penetrate the tissues readily and so reach any infected areas, and it must be tolerated in fungicidal concentrations by the host plant. Compounds which behave in this way are probably best described by the term 'systemic fungicides' and constitute a particular group of the general class of plant chemotherapeutants. There appears to be little information on the use of systemic fungicides in the treatment of leaf diseases; Polyakov (1941) claims to have increased the resistance of wheat to rust with a range of chemicals, and McNew & Sundholm (1949) have shown that certain nitrosopyrazoles will, when injected into the tomato, delay the formation of lesions caused by *Alternaria solani*.

As has already been pointed out, a successful systemic fungicide must, in addition to being fungicidal, be readily translocated and non-toxic to the host plant. There are two obvious starting-points in the search for a compound of this type: to survey known fungicides for systemic properties, or to examine the effect on fungi of compounds which might be translocated by the higher plants. In the present series of trials, a brief report of which has already been published (Crowdy & Wain, 1950), the second line of approach was chosen, and the aryloxyaliphatic acids were examined for systemic fungicidal activity.

Various workers (e.g. Hitchcock & Zimmerman (1935); Ferri (1945); Mitchell, Wood, Wolfe & Irving (1947)) have investigated the movement of synthetic growth substances within the plant. Although it has not yet been proved that these materials are translocated as such, there is much indirect evidence to show that this may be the case. The plant growth responses which follow application of certain aryloxyaliphatic acids to the soil, for example, are consistent with the hypothesis that the chemical has been translocated to the affected parts.

The growth-regulating properties of aryloxyalkylcarboxylic acids have been widely investigated but their effect on fungi has attracted little attention. Zentmyer, Horsfall & Wallace (1946) reported that 1-naphthylacetic acid reduced the growth of *Ceratostomella ulmi* in culture, and Fenner & Fate (1947) found that relatively

high concentrations of 2:4-dichlorophenoxyacetic acid stimulated the formation of large and abnormal coremia in cultures of the same fungus. Crowdy (1948) examined the effect of a number of these acids on the growth of *Nectria galligena* Bres. in culture and was led to the following general conclusions:

(1) Naphthoxy acids are more fungicidal than phenoxy acids.

(2) There is little difference in toxicity between the acetic and α-substituted propionic acids, but β-substituted propionic acids are far more toxic than either.

(3) Increasing chlorine substitution in the nucleus leads to greater toxicity.

These conclusions have been confirmed in the work now described.

A range of phenoxyalkylcarboxylic acids have been used and choice of compound has been determined largely by the absence of growth-promoting properties, preference being given to those acids with a high chlorine content in the nucleus. Selection of compounds free from growth-regulative activity was facilitated by the findings of Osborne & Wain (1949) that the aryloxy*iso*butyric acid structure is not usually consistent with activity, and of Muir, Hansch & Gallup (1949) who have indicated that nuclear substitution into the two and six positions of phenoxyacetic acids leads to inactivity.

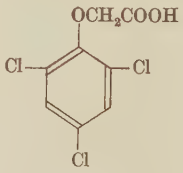
Tests were also made with 1-phenyl-3:5-dimethyl-4-nitrosopyrazole* which was one of the compounds reported as effective by McNew & Sundholm (1949). In most of the trials the test disease was chocolate spot of bean, a typical leaf disease. Preliminary trials have also been carried out against a fruit rot of tomato caused by *Botrytis cinerea* Pers.

Preparation of aryloxyalkylcarboxylic acids

Aryloxyacetic, α-aryloxyphenylacetic and β-aryloxypropionic acids were prepared by condensing the sodium derivative of the phenol in alcohol solution with the appropriate bromo ester, followed by hydrolysis. Aryloxy*iso*butyric acids were made by reacting the phenol, chloroform, acetone and solid sodium hydroxide according to the method of Bargellini (1906).

The synthesis of the three compounds which we have found most effective as systemic fungicides are described below. All were prepared by Mr C. H. Fawcett, B.Sc., of Wye College.

Preparation of 2:4:6-trichlorophenoxyacetic acid

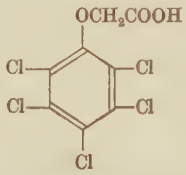


* Kindly supplied by Dr Woodcock of Long Ashton Research Station.

Sodium (3·5 g.) was added to 100 ml. absolute alcohol in a 500 ml. flask fitted with a reflux condenser. When reaction was complete 30 g. 2:4:6-trichlorophenol (m.p. 68–69° C.) was dissolved in the solution, then 25·2 g. ethyl bromoacetate added. After refluxing for 5 hr. on the water-bath, 120 ml. 10% sodium hydroxide was added and heating was continued for a further 30 min. Alcohol was then distilled off under reduced pressure, 800 ml. hot water added and the clear aqueous solution charcoaled. On acidification of the filtrate with hydrochloric acid 2:4:6-trichlorophenoxyacetic acid was obtained as a white precipitate. Yield 37 g. (m.p. 183–185° C.). The product was obtained as fine colourless needles (m.p. 184–186° C.) on recrystallization from benzene. Yield 38 g. (88% theoretical).

Equivalent. Found 256·3; $C_8H_5O_3Cl_3$ requires 255·5.

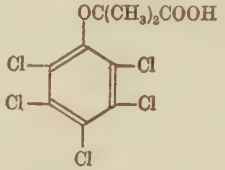
Preparation of 2:3:4:5:6-*pentachlorophenoxyacetic acid*



Sodium (0·92 g.) was added to 70 ml. absolute alcohol in a 250 ml. flask fitted with a reflux condenser. When reaction was complete 10·6 g. pentachlorophenol (m.p. 190° C.) was dissolved in the solution, then 6·7 g. ethylbromoacetate added. After refluxing for 6 hr. 40 ml. 10% sodium hydroxide was added and heating continued for another 30 min. Alcohol was distilled off, the mixture diluted with water, warmed to obtain a clear solution and acidified with 5 *N*-hydrochloric acid. The white precipitated solid was filtered, dissolved in ether and the ethereal solution was extracted four times with 2% sodium bicarbonate solution. The bulked aqueous solution (1 l.) was washed with ether, and, after distilling off dissolved ether, was cooled and acidified with hydrochloric acid. The resulting white pentachlorophenoxyacetic acid was filtered and recrystallized from aqueous methanol, giving fine white needles (m.p. 198–199° C.). Yield 5·5 g. (41% theoretical).

Equivalent. Found 322·5; $C_8H_3O_3Cl_5$ requires 324·5.

Preparation of 2:3:4:5:6-*pentachlorophenoxy*isobutyric *acid*



Pentachlorophenol (20 g., m.p. 190° C.) was dissolved in 87 ml. acetone contained in a 1 l. flask fitted with a reflux condenser. Finely powdered sodium hydroxide (18 g.) and chloroform (10·5 ml.) were then added, and, whilst swirling round by hand, the mixture was gently warmed on a sand bath for 1–2 min. until it boiled vigorously. Heating was stopped immediately and when the reaction had subsided the mixture was refluxed on the water bath for 1 hr. After standing overnight, 150 ml. water was added and excess acetone removed under reduced pressure. The mixture was made acid to congo red with hydrochloric acid and the oil which separated was taken into ether. The ethereal solution was extracted three times with 4% sodium bicarbonate solution and the bulked

aqueous solution was washed with ether, separated, and made acid to congo red with hydrochloric acid. The brown oil was extracted with ether, the extract dried over anhydrous magnesium sulphate and the ether removed. The resulting yellow-brown solid (yield 13 g.; 49% theoretical) was recrystallized twice from petroleum ether (b.p. 80–100° C.) using charcoal each time. It was thus obtained as yellow prisms, m.p. 145–146° C. (yield 9 g.).

Equivalent. Found 351·0; $C_{10}H_7O_3Cl_5$ requires 352·5.

Observations on chocolate spot of beans

Methods

In making preliminary trials with systemic fungicides the choice of a test organism is determined to a large extent by the ease with which the host can be raised under laboratory conditions, and the certainty with which the disease can be established. It was these considerations which determined the choice of chocolate spot.

Chocolate spot of bean is widely distributed, and typical symptoms follow attack by a number of species of *Botrytis*. In England two species are chiefly concerned, *B. cinerea* Pers. (Wilson, 1937) and *B. Fabae* Sardiña (Ogilvie & Munro, 1947). Both diseased conditions have been described in some detail; Wilson (1937) also includes data on the atmospheric conditions required to establish infection, namely an optimum temperature of about 15-20° C. and a humidity approaching saturation.

Both species of *Botrytis* have been used in the laboratory tests, and both formed lesions readily if the temperature was kept at about 17° C. and the atmosphere was saturated. *B. fabae* was rather less sensitive to atmospheric conditions and formed darker and more distinct lesions than *B. cinerea*, and was, therefore, more suitable for the trials.

The plants were inoculated by spraying both surfaces of the leaves with a spore suspension in a 1% dextrose solution until they were thoroughly wetted and dripping. All the plants in any one trial were sprayed with the same spore suspension, and this, combined with the thorough soaking, provided a uniform inoculation within the trial. No attempt was made, however, to standardize the spore suspensions used, so that trials could only be compared by standardizing the level of infection on the control plants. An arbitrary level of 100 was adopted for this purpose.

Early in the work it was observed that the effect of treatment was to reduce the size of the lesions rather than their number, though, in many cases, especially when lesions had developed slowly, a reduced number of lesions was noted, probably because it is only possible to count lesions that are big enough to be seen. For this reason little use of lesion counts has been made when assessing the systemic action of the fungicides; counts were, however, used to assess fungistatic action in experiments where the materials had been sprayed on the plants. In general the rate of infection was far too high to allow a total count of the lesions on the leaves, and when counts were made these consisted of comparative estimates made by counting the lesions covered by two circular microscope cover-glasses of diameter 1·25 cm.

In the earlier stages of the work the infection was assessed by scoring each leaf on an arbitrary scale ranging from 0 for an undamaged leaf to 10 for one which was severely attacked. Later this method was refined by estimating the percentage area of the leaf affected by disease, by comparison with calibrated diagrams; the diagrams used were those published by Tehon & Stout (1930) covering a range of tree fruit diseases, the types of symptom illustrated being sufficiently varied to allow easy comparison with diseased bean leaves. These two methods of assessment are essentially the same though the second has the advantage that there is some standard pattern to which reference can be made in cases of doubt. Both methods, however, suffer from the serious disadvantage that they are subjective, so that

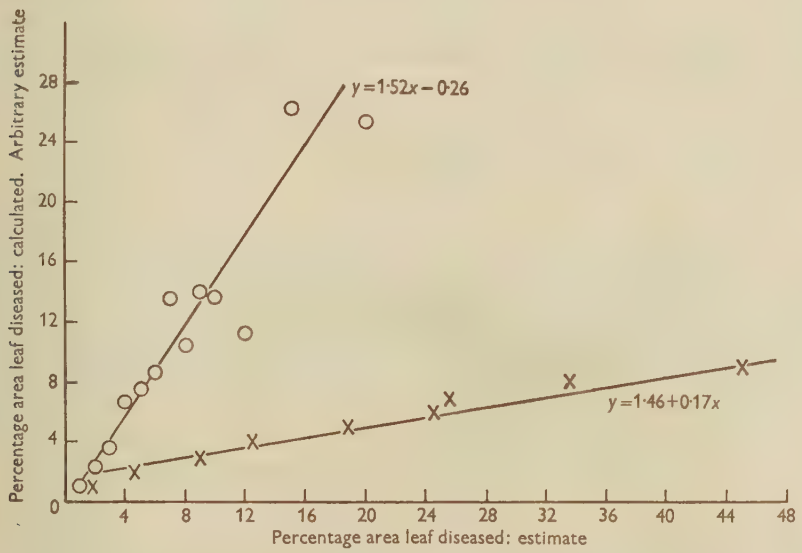


Fig. 1. Comparison of disease estimates. ○——○, percentage area estimated/percentage area calculated; ×——×, percentage area estimated/arbitrary estimate.

a wide range of error must be accepted in addition to the normal variation of the biological material. On account of this they cannot be relied upon to detect statistically significant differences smaller than about 30%, though repetition of the trials has shown in fact that these differences are genuine. In later experiments the disease on the leaves has been assessed by measuring the diameter of ten lesions on each of three or four leaves from each plant using a low-powered microscope. This method of assessment is slow and in a complex trial may involve a very large number of individual measurements; on the other hand it is relatively free from personal bias, and is not subject to errors in the inoculation technique. It is far more sensitive than the method depending on visual estimates and will show a statistical significance for

reductions in lesion diameter of the order of 10%. This technique was of special value in the assessment of infection on plants that had been treated by spraying since, under these conditions, the use of percentage area estimates was found to be unreliable. In order to preserve the continuity of the observations through the changes of technique, it became necessary to determine the relationships between the methods of assessment used; comparative data are shown in Fig. 1. As has already been stated, the assessment on an arbitrary scale and the estimate of percentage area are essentially the same method, differing only in the scale of values used. As might be expected, therefore, the agreement between the two sets of readings is reasonably good. (Correlation coefficient 0·87.) It is also possible to relate the measurements of mean lesion diameter to the estimate of percentage area affected. Thus, since the lesions are more or less circular, the area of the individual lesions can be estimated from the squares of the diameters. If this estimated lesion area is multiplied by the number of lesions on a definite area of the leaf, then an estimate is given of the total area covered by lesions in a known area of the leaf. This relationship can be summarized by the following equation

$$A = 100\,\frac{cd^2}{nD^2},$$

where A is the percentage area diseased, d is the diameter of the individual lesion, and c is the number of lesions counted in n unit areas of diameter D. Hence the area diseased is directly proportional to the lesion count and to the square of the diameter of the lesions. Fig. 1 shows the effect of comparing mean area, derived from the square of the mean diameter and the lesion count, with the percentage area affected as estimated by eye. The data were in fact very heterogeneous, but the two sets of estimated areas were highly correlated ($r = 0{\cdot}73$ for 170 degrees of freedom), and the relationship between the means was adequately expressed by the equation

$$a = 1{\cdot}52\,A - 0{\cdot}26,$$

where a is the visual, and A is the calculated estimate of the area damaged (χ^2 test showed a probability of between 0·9 and 0·8).

The relationship described above gives, within the limits of accuracy of the trials, a reasonable comparison of the percentage areas diseased for the different treatments in any one test: it does not provide an absolute measure of percentage area diseased.

No attempt was made to standardize the conditions from trial to trial, so that estimates of infection are of little value for comparing independent trials. The treatment effects have therefore been shown as percentages of the controls, and these percentages have been calculated from the direct readings of the two methods of visual estimation and from the squares of the diameters where the lesions were measured.

Although the main damage caused by the disease occurred on the leaves, infection also caused spotting of flowers and pods and shedding of the flowers. In most of the trials young plants were used, but when occasion allowed estimates of flower and fruit infection were made. The flower damage was recorded as the percentage

of unfertilized flowers shed from a number of individual inflorescences on each plant. When analysing these figures the angular transformation was used. Infection of fruit was recorded in one trial only, when estimates were made using an arbitrary scale which ranged from 0 for no infection to 5 for a fruit severely spotted.

The compounds were applied to the plants by three methods, namely through the roots, by spraying and by injection. Root application was found to be the most satisfactory. For preliminary screening very young seedlings were raised either in distilled water, or in water purified in ion-exchange resin columns, the appropriate compound being present at a concentration of 10 p.p.m., a concentration which was found, by trial, to be safe in most cases. This method was rapid and used little of the acid under test. Compounds which showed promise in the initial test were tried on plants growing in potting soil; suspensions of the acid were watered on to the pots to give a concentration of roughly 100 p.p.m. On one occasion plants growing in a garden bed were watered with a suspension of compounds at a concentration of 500 p.p.m.

Spray tests were confined to compounds showing promise in the above tests. Here, the acids were sprayed on to the beans at a concentration of 500 p.p.m., and in some trials 0·5% of a proprietary wetting agent of the sodium secondary alcohol sulphate type was also present.

Attempts were made to obtain the acid from the tops of treated plants using mild alkali and ether extraction. This extract was tested for presence of the acid by measuring its effect on the growth of *Nectria galligena*. The results obtained were inconclusive, and it would seem that work on these lines will have to await a more sensitive test for demonstrating the presence of the acid.

Development of the disease under experimental conditions

Chocolate spot is too well known to require a detailed description. Under our experimental conditions, however, the disease developed somewhat differently from the manner in which it occurs in the field. The lesions remained, on the whole, small and discrete, there being little tendency to rapid spread except where infection had killed all or part of the leaf by damaging the vascular supply, and this seldom happened in the interval between inoculation and assessment. Since the infection is always much more serious on mature than on young tissue, due allowance had to be made for this effect in analysing our results, particularly as there is a tendency for treatment to be more effective in the younger parts. In young leaves a reduction in the size of the lesions is usually noted and in very young tissue the number of lesions found is also reduced. Both these effects make a considerable difference to the estimates of percentage area affected. Damage to the flowers follows much the same course. The extent of these differences is shown in Fig. 2 which is plotted from representative data; the values for 'percentage area of leaf diseased' and 'lesion count' have been standardized by giving the most severely affected leaf an arbitrary value of 100 and calculating the remaining values in proportion.

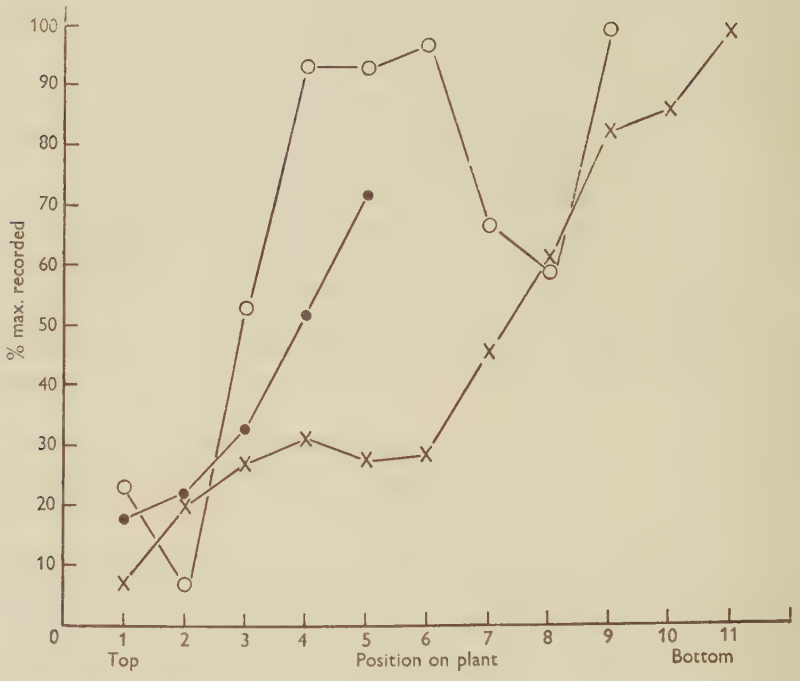


Fig. 2. Effect of age of organ on intensity of disease. ●——●, percentage flowers per inflorescence; × × , percentage area diseased per leaf. ○——○, lesion coun per leaf.

Results of the initial screening tests

Most of the compounds were screened during the early stages of the work in the period when the assay technique was being developed. For this reason some of the results were erratic. Where compounds showed early promise their activity was confirmed by repeating the tests, but it is possible that some compounds rejected in the initial screening would have shown activity in a more sensitive test. The results of these tests may, however, be of interest and are summarized below.

Compounds found to be ineffective:

2-Chlorophenoxyacetic acid
2:6-Dichlorophenoxyacetic acid
2:6-Dichloro-4-methylphenoxyacetic acid
2:6-Diiodo-4-methylphenoxyacetic acid
*2:4:6-Tribromophenoxyacetic acid
*2:4:6-Triiodophenoxyacetic acid
1:4-Dichloro-2-naphthoxyacetic acid
*3-Chloro-4-iodo-5-bromobenzoic acid
*α-Phenoxyphenylacetic acid
α-(2:4-Dichlorophenoxy) phenylacetic acid
β-(2:4-Dichlorophenoxy) propionic acid
β-(1-Naphthoxy) propionic acid
β-(2-Naphthoxy) propionic acid
β-(1-Chloro-2-naphthoxy) propionic acid

* Caused damage to the plants.

α-(2-Naphthoxy) phenylacetic and 2:4:5-trichlorophenoxy*iso*butyric acids produced a significant reduction in disease in one trial but their performance was erratic. The performance of the successful compounds is summarized in Table 1. It will be noted that all the mean reductions were highly significant. 1-Phenyl-3:5-dimethyl-4-nitrosopyrazole was included in two screening tests and gave reductions of 37 and 42%.

TABLE 1. *Reduction in chocolate spot following root treatment with acids at a concentration of* 10 *p.p.m.*

Compound used	Percentage reduction				
	No. trials	Mean	Max.	Min.	Standard error
2:4:6-Trichlorophenoxyacetic acid	15	37	62	11	4·3
2:3:4:5:6-Pentachlorophenoxyacetic acid	7	33	46	20	4·0
2:3:4:5:6-Pentachlorophenoxy*iso*butyric acid	7	27	54	7	5·3

In the tests described above the compounds were used at a strength of 10 p.p.m., but in one trial the three acids listed in Table 1 gave reductions of 45, 22 and 22% respectively when supplied at only 1 p.p.m.

Most of the compounds were harmless to the plants but more or less severe root damage was caused by 3-chloro-4-iodo-5-bromobenzoic acid, α-(2:4-dichlorophenoxy) phenylacetic acid and 2:4:5-trichlorophenoxy*iso*butyric acid. 2:4:6-Tribromo- and 2:4:6-triiodophenoxyacetic acids caused browning on the main veins of the leaves and 2:4:6-trichlorophenoxyacetic acid caused slight root damage in some experiments.

Soil treatment

The performance of the more promising of these compounds in soil was tested using bean seedlings growing in pots of sterilized potting soil. The results of the two initial trials are summarized in Table 2. The treated plants were watered with solutions of the acids instead of water prior to inoculation; in trial *A* a solution of 50 p.p.m. was used for 3 weeks, and in *B*, 100 p.p.m. for 2 weeks. The effect of both

TABLE 2. *The effect of adding the compounds to the soil on degree of chocolate spot infection*

Compound	Disease assessment (% of control)	
	Trial *A*	Trial *B*
2:4:6-Trichlorophenoxyacetic acid	55*	81
2:4:5-Trichlorophenoxy*iso*butyric acid	83†	—
Pentachlorophenoxyacetic acid	49*	91
Pentachlorophenoxy*iso*butyric acid	51*	87
1-Phenyl-3:5-dimethyl-4-nitrosopyrazole	79	103

* Significantly different from control; $P=0{\cdot}05$.
† Root damage.

these treatments was to build up a concentration of approximately 100 p.p.m. by weight of the acid in the soil. In trial *A* all the phenoxy acids produced a significant reduction in the disease with the exception of 2:4:5-trichlorophenoxy*iso*butyric acid when the weakening of the plants following root damage probably compensated for any beneficial effects of treatment. The comparative failure of trial *B* may have been due in part to the low level of infection recorded, the disease rating of the controls in *B* being only 41% of that recorded in *A*.

In order to obtain some information on how soon treatment through the roots becomes effective, potted plants were given a single treatment with 2:4:6-trichlorophenoxyacetic acid and inoculated at intervals after treatment: the results are shown in Table 3. From these results it would appear that the uptake of the compounds is rapid and, further, no marked tendency for them to accumulate in the tissues is apparent.

Table 3. *Time required for treatment with 2:4:6-trichlorophenoxyacetic acid through the roots to become effective*

Interval in days between treatment and		Disease control
Inoculation	Assessment	(%)
1	5	63
3	6	46
5	8	69

Table 4. *Effect of concentration in soil on disease control*

	Disease control (%)			
Conc. (p.p.m.) ...	2	10	20	100
2:4:6-Trichlorophenoxyacetic acid	63**	64**	70**	94
Pentachlorophenoxyacetic acid	60**	86*	59**	83*
Pentachlorophenoxy*iso*butyric acid	77**	85*	74**	69*

* Differ significantly from control; $P=0{\cdot}05$.
** Differ significantly from control; $P=0{\cdot}01$.

The results of an experiment similar to the above, designed to determine the effect of concentration of the acids in the soil on disease control are shown in Table 4. Appropriate quantities of the acids in water suspension were watered on the soil at the base of growing plants; the plants were inoculated 5 days after treatment, and assessed 3 days later. Over the limited period of the experiment the acids were effective at very low concentrations, even allowing for uneven distribution in the soil.

When considering the possible use of these compounds as systemic fungicides, their persistence in the soil becomes important, and evidence on this point was obtained in the trials summarized in Table 5. In these experiments, successive generations of bean seedlings were raised in pots of soil which had received a single initial treatment which provided a concentration of about 100 p.p.m. by weight of

compound in the soil. Each set of plants was inoculated approximately a month after sowing, and, after assessment, they were removed and a fresh set sown. The pots were kept in a greenhouse and no precautions were taken to reduce leaching. The results in Table 5 show that the compounds can remain effective in the soil for a considerable period.

TABLE 5. *Persistence of compounds in the soil*

Trial no.	Duration (days)	Compounds	Assessment no. 1	2	3	4	5
			Disease assessment as control (%)				
I	140	2:4:6-Trichlorophenoxyacetic acid	26**	75*	111	90	92
		Pentachlorophenoxyacetic acid	38**	58**	108	96	92
		Pentachlorophenoxy*iso*butyric acid	39**	67**	65*	72**	87
		1-Phenyl-3:5-dimethyl-4-nitroso-pyrazole	56**	83	100	80*	90
II	110	2:4:6-Trichlorophenoxyacetic acid	46**	52**	83**	87**	—
III	96	Pentachlorophenoxyacetic acid	133	98	70*	94*	—
		Pentachlorophenoxy*iso*butyric acid	169	74	90	105	—

* Significantly less than control; $P=0{\cdot}05$
** Significantly less than control; $P=0{\cdot}01$.

The first assessment in trial no. III (Table 5) well illustrates a difficulty encountered in assessing the results of the infection trials due to the tendency of the compounds to travel to the more actively developing parts of the plant. In this case the plants were in flower and an analysis of the percentage flowers shed on the top five inflorescences showed that pentachlorophenoxy*iso*butyric acid had produced a highly significant reduction in disease of 72%. In addition to the pot experiments a single trial was made treating the soil in a garden bed. The bean plants were fully grown when treated by watering the soil at their base with a suspension of the acids at a concentration of 500 p.p.m. Inoculation with a spore suspension was carried out about 1 week later and the plants were assessed for damage 5 weeks after treatment. The results given in Table 6 show that disease protection has resulted from the soil treatment and illustrate again that the treatment is more effective in the actively growing parts of the plant.

TABLE 6. *Treatment under garden conditions*

Compound	Disease assessment of control (%) Leaves	Pods
2:4:6-Trichlorophenoxyacetic acid	83**	63**
Pentachlorophenoxy*iso*butyric acid	100	54**

** Significantly less than control; $P=0{\cdot}01$.

Spraying of leaves

Testing the spray applications of systemic fungicides presents a number of particular difficulties: not only are the conditions governing leaf penetration little understood, but there is also the technical difficulty of distinguishing between protectant and systemic effects. The problem of differentiating between systemic and protectant effects has been approached in two ways, both subject to criticism. The first method adopted depended on applying a water spray to the treated plants. While it is probable that prolonged washing does, in fact, remove the acids from the surface of the plants, there is no easy way of testing this assumption because the acids cannot be detected in small quantities; further, washing increases the susceptibility of the plants to infection so that valid comparisons cannot be made between the washed and unwashed plants. In the second method adopted, single stems only of double-stemmed plants were sprayed and the disease was subsequently assessed on both stems. It is clear that if under these conditions a significant reduction in infection on the unsprayed half is produced the systemic effect is established. On the other hand, a negative result in this sort of trial does not necessarily mean that the compound has not been absorbed, it may simply measure a failure in translocation. With so many unknown quantities it is hardly surprising that the results of spray trials have been uncertain. Positive results have been recorded, however, and they are of interest even though there is no precise knowledge of the conditions contributing to success; Table 7 illustrates such a result. One side of each of

Table 7. *Effect of spraying with 2:4:6-trichlorophenoxyacetic acid using two-stemmed plants*

Treatment	Lesion count	Damage: mean diameter of lesion
Sprayed side:		
0·5% Wetting agent	15·1	26·1
2:4:6-Trichlorophenoxyacetic acid	9·4	21·1
Unsprayed side:		
0·5% Wetting agent	25·5	26·3
2:4:6-Trichlorophenoxyacetic acid	17·8	23·4

Differences significant at $P=0·05$; lesion count: 8·3; mean diameter: 1·38.

a number of double-stemmed plants was sprayed with 500 p.p.m. of 2:4:6-trichlorophenoxyacetic acid with 0·5% wetting agent, a corresponding number of controls being sprayed with 0·5% wetting agent alone. The plants were inoculated after 4 days, and 3 days later the number of lesions was counted and the disease was estimated from the average diameter measurements. The lesion count showed no significant differences of interest though the actual values suggest a protectant effect due to the treatment. On the other hand, the lesion measurements show definite evidence of a systemic effect; on the unsprayed side of the plant the percentage

reduction calculated from the square of the diameter of the lesion is 21, which is comparable with the results obtained from root treatment.

A very limited trial was made in a field severely affected with chocolate spot: a number of plants were sprayed with the acids at a concentration of 500 p.p.m. with 0·5% wetting agent and the percentage of flowers shed was counted on the top five inflorescences 19 days later. Pentachlorophenoxyacetic and pentachlorophenoxy*iso*butyric acids and 1-phenyl-3:5-dimethyl-4-nitrosopyrazole produced no improvement over the controls, but 2:4:6-trichlorophenoxyacetic acid produced a significant reduction of 41%.

Stem injections

Stem injection was examined as a means of introducing the acids into the plant. The least unsatisfactory method was to feed the aqueous solution from a reservoir into the base of the stem through a wick. The results presented in Table 8 illustrate that the compounds can be effective when applied in this way but the method was not used to any extent.

TABLE 8. *Stem injection with phenoxy acids*

Treatment	Damage control (%)
2:4:6-Trichlorophenoxyacetic acid	92
2:4:5-Trichlorophenoxy*iso*butyric acid	63
Pentachlorophenoxyacetic acid	57*
Pentachlorophenoxy*iso*butyric acid	42*

* Differ significantly from control.

Phytotoxicity

In using any treatment for disease control the question of damage to the treated plant is one of prime importance: this is especially so in the case of systemic fungicides because the presence of the compound within the tissues might have a more insidious effect than one which is on the surface. Visual forms of damage have already been considered, and in this section an attempt will be made to assess the less obvious damage which shows itself as a reduction in growth. Data derived from the screening trials suggest that there is little difference between 2:4:6-trichlorophenoxyacetic and the two pentachloro-acids in this respect, and that all cause a loss of about 30%. The loss in dry weight of plants raised in pots of treated soil was rather less, being of the order of 10–15%, 2:4:6-trichlorophenoxyacetic acid being the most toxic. There was no obvious difference in appearance between the plants treated with the 2:4:6-trichloro-, the pentachloro-acids and the controls.

Having regard to the effects which other compounds of this type have on the flowering and fruiting habit of treated plants, especial attention will have to be paid to the damage which may follow their use.

Discussion

The trials with the phenoxyalkylcarboxylic acids described above are essentially preliminary in nature and have been directed mainly towards demonstrating that compounds of this type are potentially useful as systemic fungicides, and developing a technique for investigating this activity.

Within the phenoxyacetic acid series, the behaviour of the compounds is much as would be expected from the growth tests with *Nectria galligena*, and it would seem that fungicidal activity can only be expected from the more highly chlorinated members of the series. The fact that there is no clear difference between the systemic effect of the trichloro- and pentachloro-acids may be due to the fact that the latter are less water-soluble, so that where the acids are present in excess, the trichloro-acid is more readily available. The failure of the β-substituted propionic acids as systemic fungicides, as has already been suggested (Crowdy & Wain, 1950), may be explained on the assumption that these acids break down in the plant to the corresponding phenol which then becomes detoxified.

The comparison between the phenoxy acids and 1-phenyl-3:5-dimethyl-4-nitroso-pyrazole is of particular interest because of the enormous difference between the performance of these types of compound as fungicides *in vitro*. Thus, the former substances induce no reduction in germination at a concentration of 100 p.p.m. at pH 4·4 or 500 p.p.m. at pH 6·3 whereas, according to McNew & Sundholm (1949), the latter prevent germination at a concentration of 1 p.p.m. This suggests that the phenoxy acids are more mobile than the nitrosopyrazole and can occur at a higher concentration in the tissues, a very valuable feature in a systemic fungicide. The tendency for the phenoxy acids to accumulate in the actively growing tissues appears to be an important factor in the performance of these compounds. Although this feature may reduce their value in treating a disease like chocolate spot which tends to be more severe in the older tissues, it might be a decided advantage in treating a disease where infection normally occurs on developing tissue. The absence of protection on old leaves, apparent even in plants which have grown from seed in treated soil, suggests that the compounds do not persist for any length of time in the tissues. It would appear that a continuous supply of the chemical must be made available to effect systemic fungicidal action.

Preliminary trials under rather unnatural conditions do not provide a reliable guide to field performance. Nevertheless, it would appear that spraying or soil application might yield encouraging results under field conditions. In this connexion it is noteworthy that spray material which does not lodge on the leaves or stems might still enter the plant through the roots: indeed, if the pot trials are any guide, the compound in the soil may provide a reservoir upon which the plant may draw for a considerable period. The low solubility of the pentachloro-acids may be a definite advantage from this point of view, since it would appear that they can cause little harm even in saturated solutions, and they are also unlikely to be readily leached from the soil.

In inoculation trials the reduction in disease due to treatment was not found to be large, but conditions were, as far as possible, adjusted to give severe infection. It is possible that protection may be more effective in the more borderline conditions often occurring naturally.

The authors would like to acknowledge the assistance of Miss P. Tozer and Miss M. Marlow for their valuable help in recording and analysing the results, and to Mr C. H. Fawcett for synthesising most of the aryloxy acids used. They are also grateful to Mr L. Ogilvie and Mrs Justham of the N.A.A.S. for providing a culture of *Botrytis fabae* and for their assistance in the two field trials.

REFERENCES

Bargellini, G. (1906). Einwirkung von Chloroform und Natriumhydrat auf Phenole in Acetonlösung. *Chem. Zbl.* **11**, 326.

Crowdy, S. H. (1948). A progress report on the effect of certain organic acids on the growth of *Nectria galligena*. *Rep. agric. hort. Res. Sta. Bristol*, 1947, 158.

Crowdy, S. H. & Wain, R. L. (1950). Aryloxyaliphatic acids as systemic fungicides. *Nature, Lond.*, **165**, 937.

Fenner, L. M & Fate, L. R. (1947). *Ceratostomella ulmi* on elm bark treated with 2:4-dichlorophenoxyacetic acid. *Phytopathology*, **37**, 925.

Ferri, M. G. (1945). Preliminary observations on the translocation of synthetic growth substances. *Contr. Boyce Thompson Inst.* **14**, 51.

Hitchcock, A. E. & Zimmerman, P. W. (1935). Absorption and movement of synthetic growth substances from soil as indicated by the responses of aerial parts. *Contr. Boyce Thompson Inst.* **7**, 447.

Horsfall, J. G. (1945). *Fungicides and their action*. Waltham, Mass.: Chronica Botanica Co.

Howard, F. L. (1941). Antidoting toxin of *Phytophthora cactorum* as a means of plant disease control. *Science* (N.S.), **94**, 345.

McNew, G. L. & Sundholm, K. (1949). The fungicidal activity of substituted pyrazoles and related compounds. *Phytopathology*, **39**, 721.

Mitchell, J. W., Wood, J. W., Wolfe, W. C. & Irving, G. W. Jun. (1947). Relative growth rates of bean and oat plants containing known amounts of a labelled plant-growth regulator (2-iodo^{131}-3-nitrobenzoic acid). *Science*, **106**, 395.

Muir, R. M., Hansch, C. H. & Gallup, A. H. (1949). Growth regulation by organic compounds. *Plant Physiol.* **24**, 359.

Ogilvie, L. & Munro, M. (1947). Chocolate spot of field beans in the south west. *Rep. agric. hort. Res. Sta. Bristol*, 1946, 95.

Osborne, D. J. & Wain, R. L. (1949). The effect of substitution into the side chain of aryloxyacetic acids on growth-regulating activity. *Proc. IInd Int. Congr. Crop Protection, London* (in the press).

Polyakov, I. M. (1941). A new method for controlling agricultural crop diseases. *Bul. Plant Prot. U.S.S.R.* no. 1, 92–9.

Tehon, L. R. & Stout, G. L. (1930). Epidemic diseases of fruit trees in Illinois 1922–28. *Bull. Ill. nat. Hist. Surv.* no. 18, 415.

Wilson, A. R. (1937). The chocolate spot disease of beans. *Ann. appl. Biol.* **24**, 258.

Zentmyer, G. A. (1942). Toxin formation and chemotherapy in relation to Dutch elm disease. *Phytopathology*, **32**, 20.

Zentmyer, G. A., Horsfall, J. G. & Wallace, P. P. (1946). Dutch elm disease and its chemotherapy. *Bull. Conn. agric. Exp. Sta.* no. 498.

(*Received* 9 *October* 1950)

THE ACTION OF MERCURY AS A SOIL FUNGICIDE

By J. R. BOOER, Ph.D., B.Sc.
Tilgate Horticultural Station, Crawley, Sussex

(With 10 Text-figures)

Metallic mercury and mercury compounds in the soil retard the growth of plants. The development of mosses and lichens is inhibited, and experimental evidence shows that the growth of toadstools on turf and the activity of ascomycetes is retarded by mercury. *In vitro*, mercury has no fungicidal action but the rate of growth of hyphae is reduced by mercury vapour.

The lack of fungicidal properties of mercury and its good performance in controlling certain soil-borne diseases are reconciled by assuming that a differential retardation disturbs the relationships necessary for infection. This assumption is supported by diagrams which treat the rates of growth of the parasite and the host as population characteristics normally distributed.

Introduction

Various uses for the inorganic mercury compounds are now well established for the control of plant diseases. The principal uses are: (1) the application of mercuric oxide or mercurous chloride mixed with fertilizer, for the control of common scab of potatoes, *Actinomycetes scabies* (Thaxt.) Gussow, described by Cunningham (1936); (2) the use of mercuric chloride or mercurous chloride for the control of club root in brassicae, *Plasmodiophora brassicae* (Woron), reported by Preston (1934, 1941); and (3) the control of white rot of onions *Sclerotium cepivorum* Berk. with mercurous chloride described by Booer (1945, 1946). Mixtures of mercuric and mercurous chlorides are also extensively used in North America for the control of the turf diseases known as brown patch, dollar spot and snow mould, as described by Monteith & Dahl (1932).

A common feature of these uses is established by the work of Booer (1944) who showed that all the mercury compounds concerned decompose, in some cases very rapidly, into metallic mercury in contact with soil. Daines (1936) had previously expressed the view that the fungicidal value of mercury compounds depends on their reduction in the soil to metallic mercury, and Preston (1941) showed that a preparation containing metallic mercury and zinc was effective against *Plasmodiophora brassicae*. It may thus be assumed that metallic mercury is the effective agent in all cases.

Metallic mercury, however, is known to be a very poor fungicide when examined by *in vitro* methods, a fact which is difficult to reconcile with its performance in the control of plant diseases. The objects of the present paper are, therefore, to examine the published results, to record hitherto unpublished data, and to formulate a theoretical explanation of the facts.

Where no reference is given, or no other location stated, the trials were made at Tilgate.

Action of mercury on plants

Zimmerman & Crocker (1934) reported that mercury vapour could cause foliage damage to numerous plants. Woodman, Brenchley & Hanley (1934) recorded that soil treatment with mercuric chloride retarded the early growth of Brussels sprouts seedlings. Gray & Fuller (1942) observed that the percentage germination of numerous vegetable seeds was not reduced by the presence of mercury in the soil, and their results also showed that subsequent growth was retarded to a slight degree. Booer (1944) used the retardation of wheat seedlings to estimate the free mercury content of soil, and stated that the fresh weights of the seedlings, above and below ground, were reduced by mercury without any obvious deformation.

During 1942 the writer made emergence tests on numerous species to determine the relative susceptibility to mercury. One classification was that the monocotyledons are only slightly retarded. Oats and barley behaved in a manner similar to wheat, as described by Booer (1944), and lawn grasses were only slightly retarded. The percentage emergence of onions was unaffected by large amounts of mercury and in 1945 a heavily replicated pot test gave the results in Table 1, which shows the mean weight per plant after 46 days growth in soil containing various amounts of mercury added as calomel.

Table 1. *Emergence, plant weights and disease losses of onions in soil containing mercury*

Mercury in soil	Emergence (%)	Mean plant wt. (gm.)	Plants dead (%)
Nil	55·4	0·153	46·7
0·0025%	59·8	0·137	18·0
0·0050%	57·4	0·131	11·9
0·0100%	59·0	0·118	15·4

In the pre-emergence stage, most of the dicotyledons appear to be more sensitive to mercury, and more variable in their reactions. The results so far available are too contradictory to permit classification, but under adverse conditions, severe pre-emergence losses may be caused by as little as 0·005% Hg in the soil. Lettuce and carrots are particularly sensitive, and in 1942 a small field trial on carrots gave a total crop failure from seed drills treated with 1 oz. 4% calomel dust per yard at sowing time. In 1942, Messrs W. J. C. Lawrence and J. Newell at John Innes Horticultural Institution, Merton, Surrey, recorded complete failure of a sowing of lettuce on land which, in a previous experiment, had received heavy applications of 4% calomel dust, a normal crop being obtained from the corresponding untreated land. This effect on carrots and lettuce was confirmed by pot tests. An exception among the dicotyledons is *Brassica oleracea* in all its varieties. Pre-emergence losses have

not been recorded in the presence of mercury in the soil, and the depression caused is comparatively slight.

In 1944, a field trial was made at the County School for Boys, Beckenham, Kent, on the control of club root in cabbages, using calomel according to the method of Preston (1942). No disease developed, and accurate yield figures showed that the treatment reduced the mean weight of the cut cabbages from 2·08 to 1·68 lb. each (sig. at $P=0{\cdot}05$). Preliminary trials on transplanting established young plants into larger pots of soil containing mercury showed that chrysanthemums were seriously retarded, but glasshouse tomatoes only slightly.

The conclusion is that mercury in the soil has a retarding effect on healthy plants. The magnitude of this effect may vary very considerably according to the species and the environment. Attention is, however, directed to the fact that the crops on which mercury is being successfully used for disease control, namely potatoes, onions, brassicae and grasses, are all retarded slightly. In most cases, mercury in the soil retards germination without causing damage, and the apparent contradiction that serious pre-emergence losses can be caused is discussed later in this paper.

The mechanism of this retardation is not yet explained, although some light has been thrown on the matter by the writer's experiments on *Sedum album* and *S. spurium* which produce new roots readily and thus eliminate the complications of retarded germination. Comparable cuttings about 2 in. long were planted in soil with and without 0·02% metallic mercury. After 3 weeks, the cuttings in the mercury-free soil had developed roots $\frac{1}{2}$–1 in. long, whereas the roots in the mercury containing soil were $\frac{1}{8}$–$\frac{1}{2}$ in. The aerial portions were similarly retarded by mercury.

When cuttings propagated in soil containing mercury are removed, washed and replanted in mercury-free soil, a normal rate of growth is resumed. Thus, the indication is that the primary action of mercury in the soil is to retard root development, any effect in the aerial parts being the sequel to root retardation. This view is supported by the fact that when seedlings, such as barley, are grown in complete darkness, the retardation of the aerial growth caused by mercury in the soil is exactly comparable with that occurring in full light.

Action of mercury on lichens and bryophytes

Specimens of *Cladonia gracilis* Willd., *C. pyxidata* Hoffm., *Peltigera canina* Willd. and *Usnea barbata* Web. were successfully raised in covered glass dishes. In similar dishes a globule of mercury, enclosed in a small calico bag, was fixed to the underside of the lid, providing a continual supply of mercury vapour. In the presence of mercury vapour, growth soon ceased, and no species of lichen eventually survived. The corresponding algae were quite unaffected and continued to grow by themselves. This was particularly noticeable in the case of *Peltigera canina*, the fronds of which changed from brown to green after about 14 days' contact with mercury vapour.

A similar effect was noted in glass dishes of unsterilized soil kept moist and warm. In untreated soil, an orange lichen, probably *Verrucaria* sp., grew freely on the sides

of the glass, whereas with 0·01% mercury in the soil there was no lichen, but a profuse growth of alga, *Protococcus* sp.

In pot experiments on lawn grasses, a plentiful crop of moss, *Funaria* sp., was obtained on the surface of the untreated soil. In soil containing 0·01% mercury no moss developed. Subsequent trials on turf showed that the application of 1 g. mercurous chloride per square yard effectively inhibited the development of moss from either spores or rhizoids for at least a year.

Viable spores of the common bracken *Pteris aquilina* completely failed to germinate in soil containing 0·01% mercury.

Action of mercury on fungi in vitro

The action of mercury compounds on fungi was examined by Parker-Rhodes (1942). Applying the statistical theory of variation to his results, he concluded that metallic mercury is not the effective agent. Dillon Weston & Booer (1935) found metallic mercury ineffective as a seed disinfectant.

The writer's preliminary tests were made with crushed seeds such as wheat or beans put into Petri dishes and moistened. The treatment consisted of placing a small globule of mercury in the dish. Treated and control dishes were incubated, and a complex mixture of fungi developed. In the presence of mercury there was a general retardation of mycelial growth, but the treatment did not appear to be selective, no species present in the controls being missing in the treated dishes. The retardation could not be measured accurately as bacterial decomposition set in, and mercury did not retard the rate of bacterial decay. The impression gained from these tests was that mercury roughly halved the rate of growth of mycelium.

Tests have also been made on nutrient agar using *Sclerotium cepivorum* and *Botrytis cinerea* Fr. and in both cases mercury reduced the rate of growth of the mycelium by about half.

Action of mercury on soil-inhabiting fungi

Actinomycetes

On the evidence that mercury retards the growth of fungi, it might be assumed that the addition of mercury to normally populated soil might result in a retardation of the development of soil-borne saprophytes.

Soil sterilization, whether by heat or by chemical agents, results in increased fertility due to the increased availability of plant foods. This effect was first recorded by Girard (1894) and Oberlin (1894) and, in the case of sterilization by heat, by Darbyshire & Russell (1907).

The most tenable explanation of the increased fertility is that advanced by Waksman & Starkey (1932), who reported that sterilization by any means is accompanied by reduced numbers of fungi and actinomycetes in the soil. They regarded the saprophytic population as unseen weeds competing with the plant

population for soil nutrients, and expressed the view that the increasing fertility produced by sterilization was the result of the reduced competition of the unseen weeds.

If increased fertility can be achieved by reducing the numbers of soil saprophytes, an increase in fertility should also be obtainable by reducing the activity of the fungus population in the soil. If mercury retards the growth of fungi, its presence in the soil should therefore result in an increase of available plant nutrients.

Numerous pot tests were made using the total crop of grass produced from a given weight of soil as the indication of fertility. Details of the technique are as follows:

'Unsterilized soil is air dried and sifted through a 6-mesh sieve. 250 g. of this soil is used in a 4 in. orchid pan previously impregnated with paraffin wax. The dimensions are such that soil fills the pot to within $\frac{1}{2}$ in. of the top. 20 ml. of the soil is removed and the remaining surface levelled. 1 g. perennial ryegrass seed is mixed with the 20 c.c. of soil, and the mixture sprinkled over the soil in the pan. For light soils, 60 ml. water is added and the total weight of the pot recorded. Additional water is added periodically to restore the original moisture content. The grass is cut to the level of the top of the pan at weekly intervals, the weight of cut grass being recorded.'

The activity of soil saprophytes can be increased by raising the status of carbon and nitrogen. The results in Table 2 are typical of the effect of mercury on soil enriched with 0·1% dried blood and 2% sawdust, and are the means of four replicates, showing the total crop of grass per pot after 9 weeks.

Table 2. *Effect of mercury on grass yield*

	Fresh weights	Dry weights
Control	2·863 g.	0·314 g.
Soil + 0·01 % Hg	3·293 g.	0·363 g.
t (n=6)	4·11	4·95

As no specific parasite was observed, the results suggest an appreciable retardation of the development of actinomycetes.

Basidiomycetes

In June 1948, various lawn treatments were applied at Tilgate, each with four replicates. One treatment was a normal lawn sand containing ferrous sulphate and ammonium sulphate. Another treatment, otherwise identical, comprised the addition of 1 g. mercurous chloride per square yard. In the autumn of 1948 the numbers of toadstools appearing in these plots were recorded weekly. The species present were *Hygrophorus niveas*, *Galera rubiginosa*, *Mycena actites* and *Nolanea staurospora*. The counts are recorded in Fig. 1, which shows the mean total of toadstools per square yard recorded at successive dates. The results show that mercury has substantially retarded the development of toadstools, without reducing the total crop.

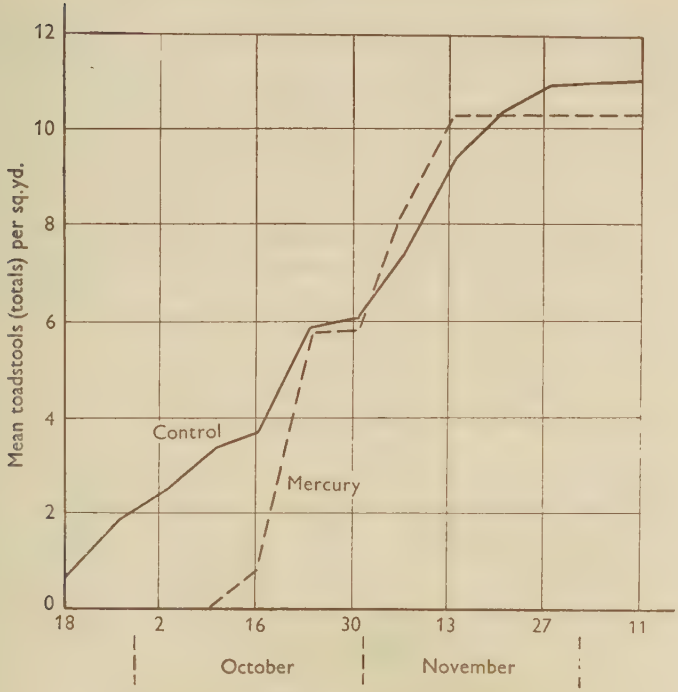


Fig. 1. Action of mercury on toadstools.

Action of mercury in disease control

The following examples from the writer's experiments are quoted to demonstrate the efficiency of mercury and mercury compounds in controlling soil-borne diseases.

A trial in 1943 on cauliflower grown under glass in pots of soil heavily infected naturally with *Plasmodiophora brassicae*, gave the following results.

	With 0·01 % mercury (%)	Without mercury (%)
Clean roots	75	4
Moderate or slight infection	25	4
Severe clubbing	Nil	92

In a pot experiment with eight replicates using soil naturally infected with *Fusarium Lini* Bolley, with and without 0·01 % mercury, the following total losses of seedlings were recorded six weeks after sowing.

	Percentage mortality
With mercury	2·00
Without mercury	64·25

The effect of mercury in the control of white rot of onions in soil naturally infected with *Sclerotium cepivorum* is shown in Table 1. It should be noted that a significant decrease in the disease control results from an excessive concentration of mercury.

Most of the published information on this subject is of this experimental type stating the varying degrees of success that were obtained. There are, however, various additional references to the adverse effects of dosages of mercury exceeding the optimum.

Cunningham (1936) quotes the results in Table 3 on the control of common scab on potatoes var. Green Mountains. Mercuric oxide mixed with fertilizer gave these results which are the means of 2 years' figures, and their importance is greatly increased by the corresponding mean yield figures in Table 4.

Table 3. *Control of common scab of potatoes*

	Scabby tubers by weight (%)			
HgO (lb./ton)	Severe	Medium	Slight	Clean
None	16·59	15·22	31·80	36·39
4	0·41	2·49	12·94	84·25
6	0·74	4·26	18·38	76·62

Table 4. *Effect of mercuric oxide on yield of potatoes*

HgO (lb./ton)	Lb./plot
4	262·43
6	256·66

Similar results have been recorded by Genereux (1942) who made trials in Quebec on the use of mercury compounds for the control of common scab of potatoes. The comparable results that he obtained are given in Table 5.

Table 5. *Yields of scab-free potatoes in bushels/acre*

HgO (lb./acre)	1940	1941	1942	Mean
0	105·9	53·8	84·6	81·4
2½	97·1	64·5	103·2	88·2
5	100·1	68·6	92·9	87·2
10	87·8	63·7	82·9	78·1

An important aspect common to all the quoted results is that no treatment gives complete freedom from disease, a fact to which various authors refer. Cunningham (1936) states that the treatment '. . . reduces the infestation of Common Scab of the potato'. Preston (1941) observes '. . . that none of the substances used gave complete immunity from club root infection'. Booer (1946) referring to 3 years' work on onions, mentions that '. . . there is no instance of the disease being completely controlled'. With the wide range of conditions under which the three methods have been tested, it is improbable that such a result would occur by chance.

A THEORY OF THE DISEASE-CONTROLLING ACTION OF MERCURY

The various results that have been quoted may be summarized by stating the requirements of any theory of the action of mercury as a soil fungicide. These are:

(1) Mercury is a poor fungicide.

(2) Its use in the soil can produce a high degree of disease control.

(3) Complete control of disease has not been recorded.

(4) Mercury in the soil retards the growth of both plants and fungi.

(5) In excess of the optimum dosage both the disease control effect and the yield may be diminished.

Fig. 2. Normal distribution of rate of plant growth.

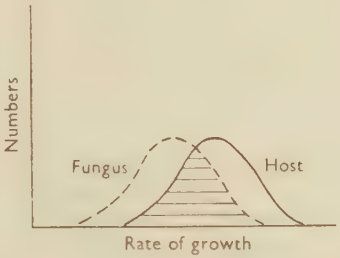


Fig. 3. Partial infection by retarded fungus population.

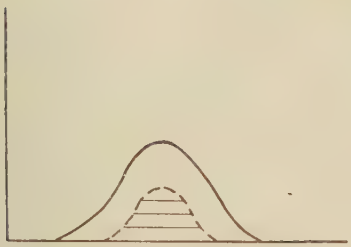


Fig. 4. Partial infection by small fungus population.

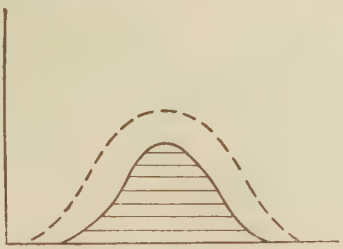


Fig. 5. One hundred per cent infection by large fungus population.

Many authors have stressed the necessity for considering the parasite and the host simultaneously. In the case of root-disease fungi this view has been summarized by Garrett (1944) (p. 29) who, commenting on the work of de Jong (1933), states that the results 'underline the essential fact that infection is a "struggle" between the offensive forces of the fungus and the defensive forces of the root...and the issue of the "struggle" is likely to be decided by the extent of the reserves available on either side'. On the present evidence it would appear that the favourable influence of mercury on the 'struggle' must be due to the fact that it retards the causal fungus more than the host. In order that this effect may be translated into disease control, it is necessary to assume that mercury disturbs the relationship

between the host and the parasite, possibly a symbiosis, which is the pre-requisite of infection.

With some plant diseases it is already known that certain changes in the host are necessary before infection can take place, and that the degree of infection can be related to the size or composition of the vulnerable cells. Such changes in the host are usually related to the rate of growth, and for the purpose of presenting this theory the 'rate of growth' is used as a generalization expressing the rate at which a vulnerable condition develops. Whatever the 'rate of growth' may be, it can be regarded as a populational characteristic distributed normally as shown in Fig. 2.

The development of the aggressive organ of the parasite may also be regarded as a normally distributed populational characteristic, and in this case it is probable that the rate of growth is the correct description.

By selecting suitable units, the same abscissae can be used for both populations and the conditions necessary for partial infection are shown in Fig. 3. The shaded area is a close mathematical approximation to the numbers of plants in which the conditions necessary for infection have been fulfilled and thus the percentage infection is the ratio of the shaded area to the area representing the whole population. Fig. 4. illustrates a partial infection caused by a small fungus population, and Fig. 5 a 100% infection by a relatively omnivorous parasite such as *Botrytis cinerea*.

Application of the theory

The theory can be used to show that when the retardation of the fungus is greater than that of the host, a measure of disease control will take place. Fig. 6*a* shows what could be the state of affairs in the controls of an imaginary experiment. The infection is 75%. In Fig. 6*b* the effect of the mercury treatment is shown as retarding the plants slightly, and the fungus still more, thus reducing the infection to 12%. These figures agree closely with those quoted by Booer (1946) for the control of White Rot in transplanted onion seedlings. The mercury treatment reduced the infection from 74·3 to 11·9%.

The analysis of results on the control of diseases where multiple infections may be involved, such as club root in brassicae, entails certain arbitrary assumptions, justified by the fact that the disease classifications used by the investigators are empirical. Both Cunningham and Preston use the classifications 'severe', 'moderate', and 'slight', which are determined by arbitrary scales. By assuming that 'severe' infection occurs when the ratio of fungi to plants is above some arbitrary limit, as 0·7, and 'moderate to slight' infection occurs when the ratio is between 0·7 and 0, the results of an imaginary experiment can be depicted as shown in Figs. 7*a*, *b*, in which the severity of infection is indicated by the depth of hatching. The figures show an infection in the controls of about 65% severe, and 15% moderate, with 20% healthy. The treatment resulted in a reduced infection of 30% severe and 10% moderate, with 60% healthy. These figures bear a marked

resemblance to many of the results quoted by Preston (1941, 1942), and it is obvious that by suitable adjustment of the graph, the results of Cunningham (1936) could be reproduced.

The fact that complete disease control is not obtained can be explained if the

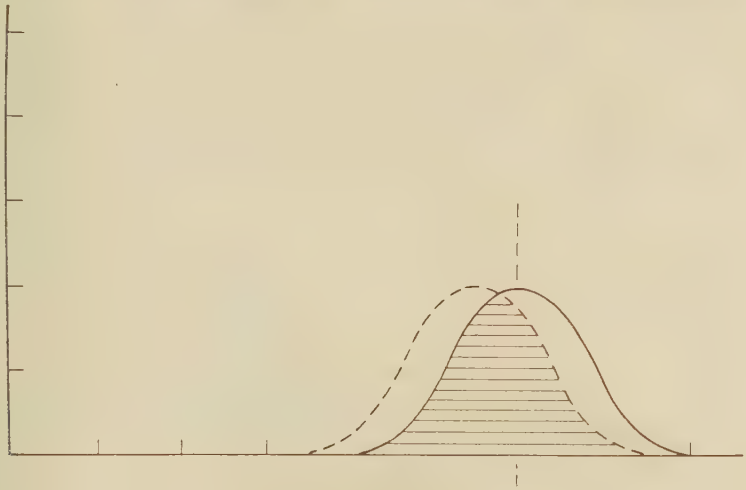


Fig. 6*a*. No treatment.

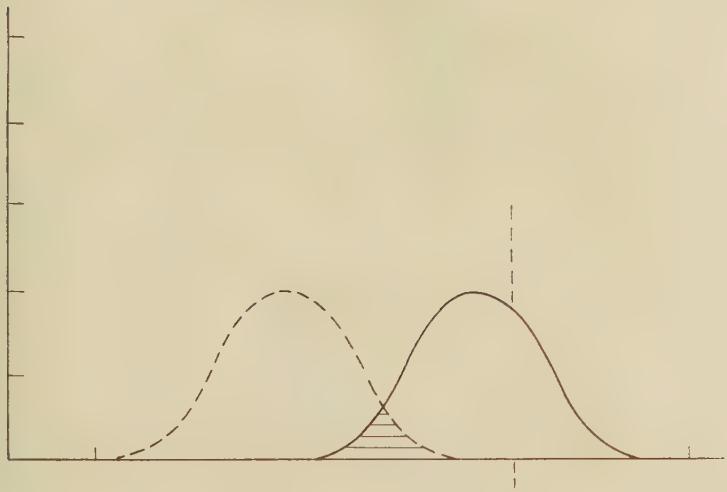


Fig. 6*b*. Effect of mercury.

maximum differential retardation is insufficient to separate completely the two population curves. Booer (1944) has shown that the retardation of wheat seedlings is within certain limits proportional to the logarithm of the mercury concentration.

This suggests that the complete curve relating retardation to mercury concentration is sigmoid in form, rising to a maximum as shown by the continuous line in Fig. 8. If the corresponding curve for the retardation of the fungus takes the same form,

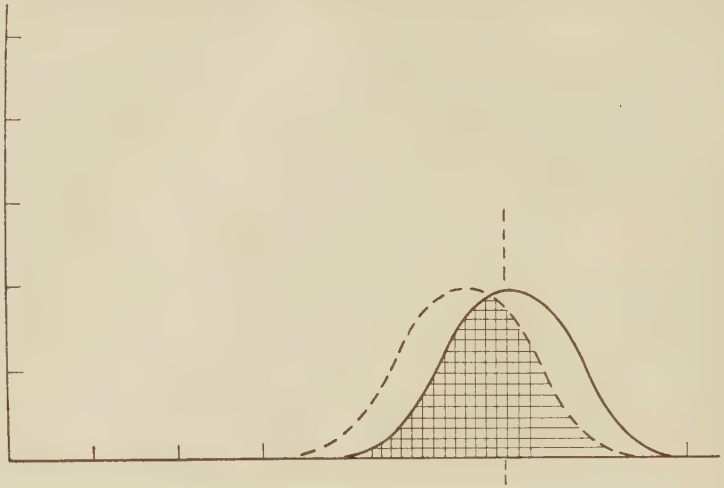


Fig. 7*a*. Effect of mercury.

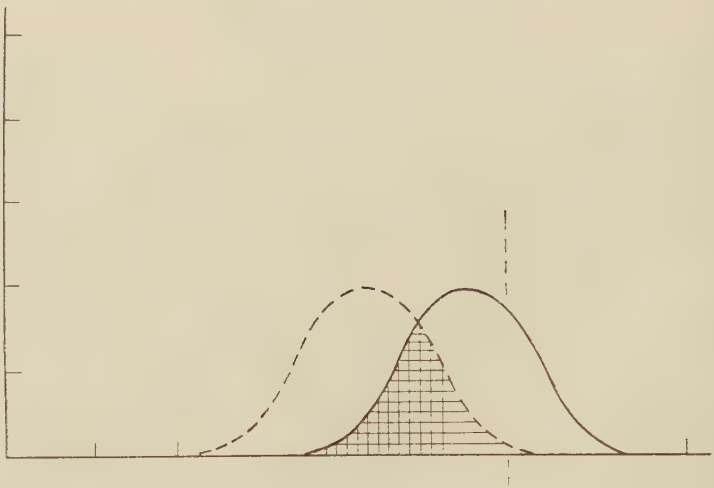


Fig. 7*b*. No treatment.

the maximum difference *AB* is reached at a certain mercury concentration, above which no improvement in disease control will result. Fig. 9 illustrates the circumstances in which increasing the mercury concentration above the optimum may result in poorer disease control.

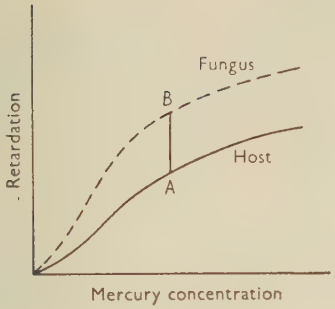


Fig. 8.

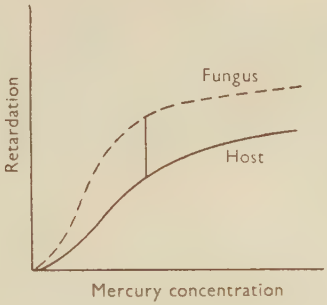


Fig. 9.

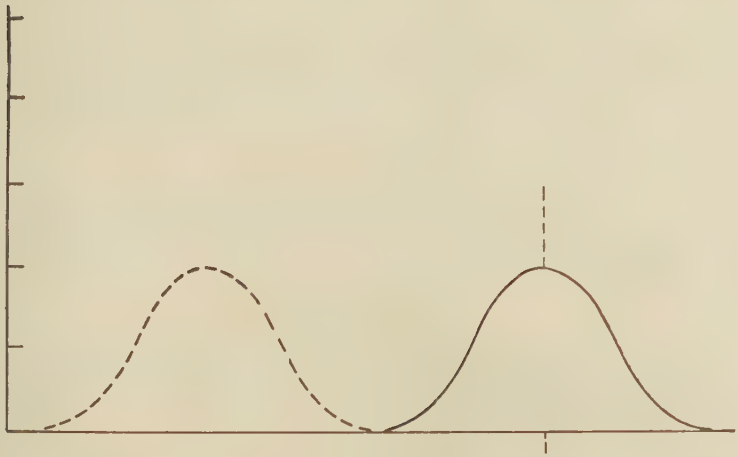


Fig. 10*a*. No treatment.

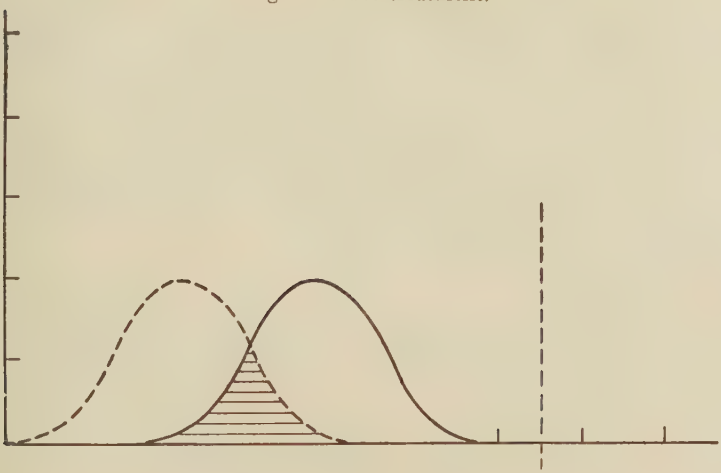


Fig. 10*b*. Effect of mercury.

The occasional pre-emergence losses apparently caused by mercury may be explained by the presence of low-temperature parasites such as *Pythium* spp. The retardation to the growth of the plant caused by mercury is similar to that caused by low temperatures, and if the fungus is not retarded to the same extent, infection will occur. This is illustrated in Figs. 10*a*, *b*.

Conclusion

The theory indicates the possibility of controlling soil-borne diseases with any agent which, although not lethal to the causal fungus, will bring about a retardation of its rate of growth greater than the retardation exerted on the rate of growth of the host plant.

In the case of mercury, the known facts, and the theoretical explanation of them, clearly indicate that all preliminary trials should be made with at least two mercury concentrations. If the higher concentration gives a poorer result, the theory shows that this is not necessarily discordant.

The author is indebted to Prof. A. E. Muskett for supplies of soil infected with flax wilt, and to Messrs W. J. C. Lawrence and J. Newell for permission to refer to unpublished results.

REFERENCES

Booer, J. R. (1944). The behaviour of mercury compounds in soil. *Ann. appl. Biol.* **31**, 340.

Booer, J. R. (1945). Experiments on the control of white rot (*Sclerotium cepivorum* Berk.) in onions. *Ann. appl. Biol.* **32**, 210.

Booer, J. R. (1946). Further experiments on the control of *Sclerotium cepivorum* Berk. *Ann. appl. Biol.* **33**, 413.

Cunningham, H. S. (1936). The addition of mercury compounds to the fertilizer as a control for common scab of the potato under Long Island conditions. *Amer. Potato J.* **13**, 100.

Daines, R. H. (1936). Fungicidal action of mercury in soils. *Phytopathology*, **26**, 90.

Darbyshire, F. V. & Russell, E. J. (1907). Oxidation in soils and its relation to productiveness. Part II. The influence of partial sterilization. *J. agric. Sci.* **2**, 305.

Dillon Weston, W. A. R. & Booer, J. R. (1935). Seed disinfection. I. An outline of an investigation on disinfectant dusts containing mercury. *J. agric. Sci.* **25**, 628.

Garrett, S. D. (1944). *Root disease fungi*. Waltham, Mass.: Chronica Botanica Co.

Genereux, H. (1942). Disinfection of soil with mercury compounds as preventative for common scab of potatoes. *Ann. l'Acfas*, **8**, 116.

Girard, A. (1894). *Bull. Soc. nat. Agric. Fr.* **54**, 356.

Gray, N. E. & Fuller, H. J. (1942). Effects of mercury vapour on seed germination. *Amer. J. Bot.* **29**, 456.

Jong, W. H. de (1933). Het parasitisme van *Rigidoporus microporus* = *Fomes lignosus*, bij *Hevea brasiliensis* (with English summary). *Arch. Rubbercult. Ned.-Ind.* **17**, 83.

Monteith, J. M. & Dahl, A. S. (1932). Turf diseases and their control. *Bull. U.S. Golf Ass.* (Green Section), **12**, 85.

Oberlin, C. (1894). *Bodenmüdigkeit und Schwefelkohlen* (Mainz).

Parker-Rhodes, A. F. (1942). Studies on the mechanism of fungicidal action. IV. Mercury. *Ann. appl. Biol.* **29**, 404.

Preston, N. C. (1934). The control of club root (finger and toe) in cauliflowers. *J. Minist. Agric.* **41**, 329.

Preston, N. C. (1941). Experiments on the control of club root of brassicae in gardens and allotments. A summary of six years' trials. *Ann. appl. Biol.* **28**, 351.

Preston, N. C. (1942). Club root disease. Calomel as a preventative. *Fruitgrower*, **93**, 202.

Waksman, S. A. & Starkey, R. L. (1923). Partial sterilization of soil, microbiological activities and soil fertility. I. *Soil Sci.* **16**, 137.

Woodman, R., Brenchley, G. H. & Hanley, F. (1934). Soil treatment for Brassica I. The effect of sterilization of the soil by mercuric chloride on the seedling growth of Brussels sprouts. *J. Soc. chem. Ind., Lond.*, **53**, 35T.

Zimmerman, P. W. & Crocker, W. (1934). Plant injury caused by vapours of mercury and compounds of mercury. *Contr. Boyce Thompson Inst.* **6**, 167.

(*Received* 28 *July* 1950)

THE SUBEPIDERMAL FUNGI OF CEREAL GRAINS

II. THE NATURE, IDENTITY AND ORIGIN OF THE MYCELIUM IN WHEAT

By M. B. HYDE and H. B. GALLEYMORE
Pest Infestation Laboratory, Slough, Bucks

(With Plate 8 and 2 Text-figures)

An internal fungal mycelium between the epidermis and cross-layer cells in normal wheat grains is shown to consist of extracellular septate hyphae forming a network on the inner surface of the epidermis, whose cells are occasionally penetrated. The mycelium, which is first observed at a comparatively late stage of maturation of the grain, usually remains as a loose network, although sclerotia and plate-like structures suggestive of drought forms are occasionally present. These appearances support the view, derived from comparison of the drying rates of grain under various climatic conditions, that the spread of the subepidermal mycelium is finally restricted by desiccation beneath the epidermis during the drying out of the ripening grain.

Cultures from surface-sterilized grains of Bersée wheat have shown that the most common subepidermal fungus is *Alternaria tenuis* (in 64·4% of the grains). Bacteria (28·8%), *Mycogone* sp. (?) (7·7%), *Cladosporium herbarum* (5·8%), *Pullularia pullulans* (4·8%), *Fusarium* sp. (1·9%), *Botrytis cinerea* (1·9%) and *Stemphylium botryosum* (0·9%) were also isolated.

The subepidermal mycelium apparently arises either from systemic infection of the wheat plant or from fungal spores and hyphae present on the outside of the developing grains and among the dead floral parts.

Introduction

The paper by Oxley & Jones (1944) was followed by brief accounts of internal fungi in cereal and other seeds by Schwartz-Kraepelin (1947), Whitehead, Thirumalachar & Dickson (1948) and Bose (1950). They have confirmed the statements in earlier papers (Bolley, 1913; Niethammer, 1939; Marcus, 1942) that a subepidermal mycelium is present in healthy, mature grains of wheat and other cereals, but one or two of them have contained ambiguous and even misleading statements. Schwartz-Kraepelin (1947) appears to have been misled by being unable to find actual penetration of the epidermal cells by fungal hyphae into assuming that the internal hyphae, which 'arise in cell layers which are digested during development of the seeds', 'are formed shortly before maturation without germination from spores being recognizable' (translation), a process implying spontaneous generation, a view also held by Elfving (1941) for the origin of certain bacteria in seeds. Bose's (1950) suggestion that there may be a possible connexion between rust resistance and the subepidermal mycelium in wheat is not supported by the almost universal presence of the internal mycelium (Hyde, 1950) in both immune and susceptible varieties (as described by Manners, 1950).

The present investigation was undertaken to investigate further the nature of the subepidermal mycelium, its position within the grain, its identity and its time and mode of entry.

Nature of the subepidermal mycelium

Position within the grain

The very varied and somewhat vague descriptions in the literature of the location of the internal mycelium (Marcus, 1942, 'beneath the pericarp'; Schwartz-Kraepelin, 1947, 'on the inner side' of the pericarp; Bose, 1950, 'just beneath the testa within the tegmen of the grain') are not in agreement with the observations detailed below. As discrepancies probably arise because of differences in terminology, a brief description of the covering of the wheat caryopsis seems desirable.

The outer covering of a mature wheat grain consists of: (1) an outer epidermis (the exocarp), generally one cell in thickness, composed of elongated cells running parallel to the long axis of the grain; (2) a mesocarp or layer of crushed cells; and (3) an inner pericarp (the endocarp) comprising two layers of cells, the so-called cross-layer cells, whose long axes are at right angles to those of the epidermal cells, and the tube cells. The latter do not form a compact layer and are sometimes difficult to recognize in the mature grain. This multi-layered pericarp encloses the testa.

The mesocarp has generally disintegrated by the time the grain is mature, although isolated cells may remain intact, and others form a transparent, amorphous mass, Härdtl's (1935) *Hyalinschicht*, which should not be confused with the true 'hyaline layer' of the testa itself. In the cavity formed between the epidermis and the cross-layer cells by the breakdown of the mesocarp the subepidermal mycelium develops. It is possible that it obtains nutriment from the disintegrating mesocarp cells.

The cells of the aleurone layer surrounding the endosperm appear to be free from fungal hyphae. This is confirmed by Marcus (1942). Peklo (1913), however, believes that the aleurone layer is of fungal origin, and Niethammer (1939) considers that the development of fungi is correlated with the production of growth substances in the aleurone layer. None of the observations of the present authors lends any support to either of these views, and the illustrations of Peklo and Niethammer are considered to be unconvincing.

Previous investigations (Hyde, 1950) have shown that in temperate climates the mycelium frequently extends over the greater part of the inner surface of the epidermis, being particularly abundant in areas where the epidermis is loose, i.e. at the two ends of the grain, along the sides of the crease and in the 'breast' region. When infection is less heavy, the mycelium may be restricted to limited areas, particularly below the beard hairs and to a lesser extent at the embryo end of the grain.

Appearance of the mycelium

If a wheat grain is soaked for a short time in cold water the epidermis may be stripped off intact and carries with it virtually the whole of the subepidermal mycelium. Only a very few small fragments of hyphae remain attached to the cross-layer cells. Unstained mycelium is scarcely distinguishable from the pale, yellowish brown cells of the epidermis, but after warming for 5–10 min. in aniline blue (0·2%) in 66% lactic acid, followed by differentiation in warm lactic acid the fungal hyphae normally appear deep blue, while the epidermal cells remain unstained.

The mycelium normally consists of septate hyphae branching repeatedly in all directions to form an extensive network (see Pl. 8, figs. 1 and 2). Sometimes the hyphae have only a few septa, so that the cells are very long (Pl. 8, fig. 1). In another equally common type (Pl. 8, fig. 2) the cross-walls are more abundant, resulting in shorter cells. The mycelium is usually extracellular, but intracellular hyphae are occasionally found filling individual cells of the epidermis. The hyphal cell walls are generally thin and colourless, but sometimes a darker mycelium occurs, with brownish cell walls (Pl. 8, fig. 3). Other less common types include some with very fine hyphae, and others with short, broad cells, the mycelium branching frequently to form a flat, plate-like structure, staining only faintly with aniline blue (Pl. 8, fig. 4).

Generally the hyphae occur as a wide-meshed network although sclerotia are sometimes present. Of 5000 grains examined from a wide range of countries (Hyde, 1950), only two or three showed spores and these in very small numbers. Other investigators, e.g. Marcus (1942), have also commented on the absence of spores *in situ*.

Identity of the fungi

The considerable diversity of growth forms found, sometime several different types in one grain, indicates that several species of fungi are present. These were identified from cultures of the internal mycelium from surface-sterilized grains. As the epidermis is made up of only one layer of cells, it was necessary to employ the following procedure, which satisfactorily removed surface contaminants without damaging the subepidermal mycelium.

The grains were wetted for 20 sec. in 70% alcohol, immersed for a further 20 sec. in a 1:1000 solution of mercuric chloride, and washed in two changes of sterile distilled water. Portions of the epidermis were stripped from each grain and plated out on to 2% plain agar and 2% agar containing either 3% malt extract or an extract from ground wheat. The cultures were incubated at 25° C. for about 7 days, by which time the developing fungi could be identified or subcultured for further examination or for induction of sporulation. The following list gives the micro-organisms isolated in this manner from the subepidermal position in Bersée wheat, together with the percentage, in brackets, of the examined seeds infected with each micro-organism: *Alternaria tenuis* (64·4), *Bacteria* (28·8), *Mycogone* sp. (?)

(7·7), *Cladosporium herbarum* (5·8), *Pullularia pullulans* (4·8), *Fusarium* sp. (1·9), *Botrytis cinerea* (1·9), *Stemphylium botryosum* (0·9).

It is possible that the above list may include some fungi which were external to the grain, although the absence of the usual contaminants makes this unlikely, or that it may exclude some internal fungi which were regularly killed by the technique adopted.

The preponderance of *Alternaria* as an internal fungus is also noted by Bolley (1913), Mead (1933), Marcus (1942), Groves & Skolko (1944*b*) and Bose (1950). Other fungi that have been found include species of *Macrosporium*, *Helminthosporium*, *Colletotrichum*, *Fusarium* and *Cephalothecium* (Bolley, 1913); *Helminthosporium sativum*, *Penicillium* sp. and *Fusarium* sp. (Mead, 1933); *Mucor hiemalis* and *Trichoderma Koningi* (Niethammer, 1939); *Cephalosporium acremonium* (Marcus, 1942); *Fusarium* sp. (Gordon 1944); and *Stemphylium botryosum* (Groves & Skolko, 1944*a*). These authors did not always state the exact location of the fungi which may not therefore all be from the subepidermal region.

Entry into the grain

Time of entry

Observations for three seasons of very different weather conditions (1948, 1949 and 1950) have been made on the entry of the subepidermal fungi into developing wheat grains. Morphological growth stages of the ears have been distinguished arbitrarily, and it has been possible to correlate the later of these stages with the moisture content of the ripening grains (see Table 1).

Although there were slight differences between varieties Table 2 shows that all remained free from subepidermal mycelium until a comparatively late stage of development, about stages 8–9 (as defined in Table 1). Table 2 also shows that the date of entry varied according to the weather during the ripening period, being earlier in the dry season of 1949 than in the wet summer of 1950. The mycelium, small in amount when first seen, appeared simultaneously in about 30% of the grains of any particular variety, from which samples had been taken daily during the development period. At this stage the grains had reached full size and maximum dry weight, and had begun to dry out. During the first few days after its appearance the mycelium spread rapidly beneath the epidermis, but soon reached an amount* which remained constant for the remainder of the development period and after harvest, by which time 100% of the grains were infected.

The increase in amount of mycelium occurred while the moisture content of the grain was falling rapidly (see Text-fig. 1) and presumably would have continued indefinitely if it had not been stopped by insufficient humidity in the subepidermal space. Evidence that low humidity is the factor which checks development of the

* A method of assessing amount of subepidermal mycelium is described in the first paper of this series.

subepidermal mycelium is given by the fairly frequent occurrence of a flat, plate-like type of growth (p. 350 and Pl. 8, fig. 4). This thallus-like structure is very similar to the *Kummerformen* or stunted forms produced at the lower limits of humidity by various fungi, as described and illustrated by Heintzeler (1939).

The amount of subepidermal mycelium in a grain will clearly depend largely on the duration of favourable humidity and thus will vary inversely with the rate of

TABLE 1. *Morphological growth stages and moisture content of the grain in developing wheat ears*

Stage	Morphological characteristics	Moisture content of grain (%)
6*	Plant about full height; some undeveloped spikelets have lost their chlorophyll; grain about full size and at 'milk' stage	60–70
7	Many glumes yellow; endosperm at 'soft dough' stage; cells of cross-layer yellow except near crease; mesocarp disintegrating	*c.* 50
8	All chlorophyll gone from glumes and cross-layer cells; grains, especially at top of ear, orange in colour but still soft; final dry weight reached; most of contents gone from cells of epidermis, which is now thin and papery; mesocarp almost gone	35–40
9	Glumes open, so that grains are visible; most grains beginning to harden	*c.* 30
10	Grains fairly hard; epidermis can just be stripped from grains without preliminary soaking	25–30
11	Grains hard; need soaking before epidermis can be removed	20–25
12	Ripe and ready for harvest	15–20
13	'Dead ripe'	In equilibrium with atmospheric humidity

* Earlier stages are not included in this table.

TABLE 2. *Stage at which subepidermal mycelium is first seen*

1948			1949			1950		
Variety	Stage	Date*	Variety	Stage	Date*	Variety	Stage	Date*
Bersée	9	26	Bersée†	8	7	Bersée‡	7+	13
Yeoman‡	8–9	23	Bersée	8–9	23	Yeoman‡	8	20
Bersée‡	8–9	23	Vilmorin	8–9	25	Bersée	8	27
Holdfast‡	9	26	Yeoman	8–9	25	Yeoman	8	28
Squareheads Master‡	9	26	Atlé	8–9	27			
Pilot‡	10	29	Squareheads Master	9–10	27			
Generosity‡	10	29						

* All dates in July.
† Autumn sown; otherwise, spring sown.
‡ Grown at Heston, Middlesex; otherwise at Slough.

drying of the grain. Hyde (1950) has noted the correlation between pre-harvest atmospheric humidity and the amount of subepidermal fungus in wheats from many parts of the world. Further evidence is provided by a comparison of the

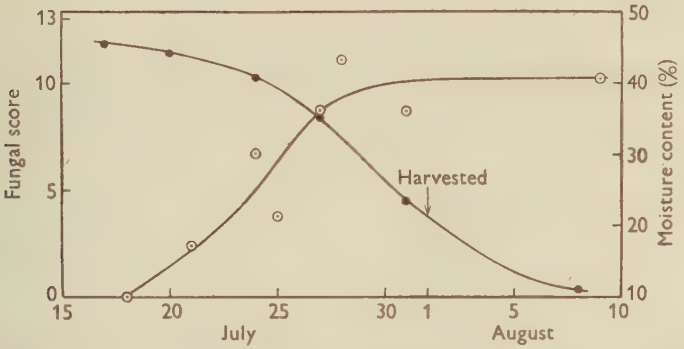


Fig. 1. Relation between pre-harvest moisture content and fungal score in Yeoman autumn-sown wheat (1950 crop). ●—●, moisture content; ⊙—⊙, fungal score.

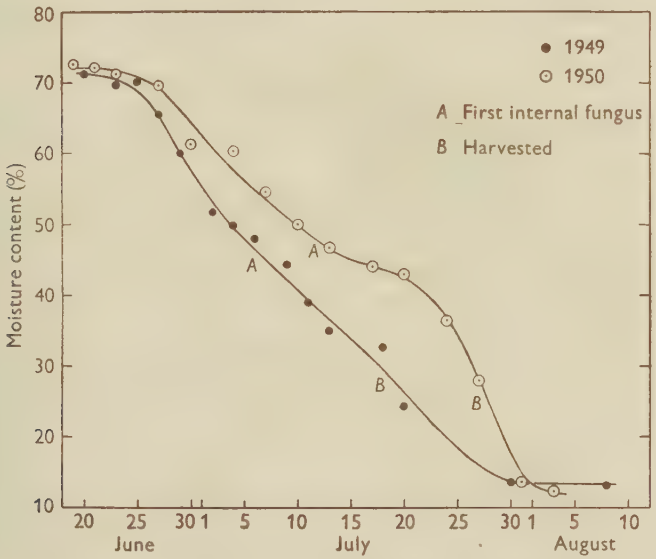


Fig. 2. Comparison of drying rates of maturing wheat grains in 1949 and 1950.

drying rates and final amounts of fungus in autumn-sown Bersée wheat grown at the Pest Infestation Laboratory during the very dry season of 1949 and the wet one of 1950. Text-fig. 2 shows that in 1949 the moisture content of the grains fell rapidly and continuously after the fungi had first entered the grains, whereas in 1950 the

moisture content remained very high for over a week after fungal entry. The resulting amounts of subepidermal fungus, expressed as 'fungal scores' determined as described in the previous paper of this series (Hyde, 1950) were: 1949, 4·9; 1950, 9·3.

Mode of entry

The method of entry of the subepidermal mycelium is not yet established. One possible explanation is that the mycelium develops from the germination of air-borne spores on the outside of the immature wheat grains exposed by the opening of the glumes. However, no instance of actual penetration of the epidermis has been seen, except for occasional *outgrowths* of hyphae through the openings of stomata near the crease at the beard end of the grain. Support for this theory is however given by the very frequent occurrence of similar types of hyphae both on the outside and within the epidermis of the same grain, especially among the beard hairs. Many of the spores found on the outside of the grains, particularly in the beard region, were those of *Alternaria tenuis*, *Fusarium* spp. and other species commonly isolated from inside the grains.

During the development of the ear these and other fungi such as *Helminthosporium* spp. and *Erysiphe* spp. develop abundantly on the dead remains of the stamens and stigmas, first becoming noticeable at about growth-stage 6. These dead floral parts remain closely associated with the developing grains and thus provide a possible source of infection through the epidermis. Observations over several years also show that the majority of grains have far more internal mycelium at the beard end than at the basal embryo region.

Another possibility is that the subepidermal fungi develop from a systemic mycelium as has been described for similarly situated fungi in the seeds of *Lolium* spp. (Sampson, 1937; Freeman, 1903). To investigate this, a large number of flowering stems of Bersée wheat was examined over a period of 2 months up to and including harvest by cutting six quarter-inch sections from the basal internode of each plant, and six from the stem immediately below the inflorescence. The sections were surface-sterilized, washed and plated out using a similar technique to that used for the grains (p. 350). The results from 70 stems (25 autumn-sown plants and 45 spring-sown) are given in Table 3.

The similarity between the floras obtained from stem sections and from grains is evident both in the kind and in the relative abundance of the micro-organisms isolated. This strongly suggests that the subepidermal mycelium may be developed from a systemic 'infection' of the wheat plant. It is possible, however, that the similarity of the micro-organisms isolated from stems and grains is, at least in part, a consequence of the sterilizing technique common to both.

A dual origin for the subepidermal mycelium is therefore suggested. It may arise either from a systemic infection or from fungal spores and hyphae present on the outside of the developing grains and among the dead floral parts.

TABLE 3. *Micro-organisms isolated from wheat stems*

	Number of plants from which each species was isolated	
	In basal sections	*In upper sections*
Alternaria tenuis	40	43
Pullularia pullulans	10	33
Bacteria	20	32
Cladosporium herbarum	10	26
Fusarium sp.	2	8
Yeasts	1	9
Cephalosporium sp.	9	1
Mycogone sp.	1	7
Botrytis cinerea	2	5
Chaetomium sp.	—	1
Penicillium sp.	—	3
Stemphylium botryosum	—	1

This investigation formed part of the programme of work of the Pest Infestation Laboratory and this account is published by permission of the Department of Scientific and Industrial Research. Miss F. J. Bowyer and Mr D. Budd assisted in the experimental work. The authors are indebted to Mr J. H. Hammond for the preparation of the photomicrographs.

REFERENCES

BOLLEY, H. L. (1913). Wheat. Soil troubles and seed deterioration. *Bull. N. Dak. agric. Exp. Sta.* no. 107, 96 pp.

BOSE, S. R. (1950). Problem of wheat rust. *Nature, Lond.*, **166**, 352.

ELFVING, F. (1941). Elternlose Bakterien. *Comment. biol., Helsingf.*, **9**, 1.

FREEMAN, E. M. (1903). The seed fungus of *Lolium temulentum* L., the Darnel. *Philos. Trans.* **196**, 1.

GORDON, W. L. (1944). The occurrence of *Fusarium* species in Canada. I. Species of *Fusarium* isolated from farm samples of cereal seed in Manitoba. *Canad. J. Res.* C, **22**, 282.

GROVES, J. W. & SKOLKO, A. J. (1944*a*). Notes on seed-borne fungi. I. *Stemphylium*. *Canad. J. Res.* C, **22**, 190.

GROVES, J. W. & SKOLKO, A. J. (1944*b*). Notes on seed-borne fungi. II. *Alternaria*. *Canad. J. Res.* C, **22**, 217.

HÄRDTL, H. (1935). Zur Mikroskopie der Fruchthülle unser wichtigsten Getreidearten. *Z. Untersuch. Lebensmitt.* **69**, 113.

HEINTZELER, I. (1939). Das Wachstum der Schimmelpilze in Abhängigkeit von den Hydraturverhältnissen unter verschiedenen Aussenbedingungen. *Arch. Mikrobiol.* **10**, 92.

HYDE, M. B. (1950). The subepidermal fungi of cereal grains. I. A survey of the world distribution of fungal mycelium in wheat. *Ann. appl. Biol.* **37**, 179.

MANNERS, J. G. (1950). Studies on the physiologic specialization of yellow rust (*Puccinia glumarum* (Schm.) Erikss. & Henn.) in Great Britain. *Ann. appl. Biol.* **37**, 187.

MARCUS, O. (1942). Über das Vorkommen von Mikroorganismen in pflanzlichen Geweben (nach Untersuchungen an Früchten und Samen). *Arch. Mikrobiol.* **13**, 1.

MEAD, H. W. (1933). Studies of methods for the isolation of fungi from wheat roots and kernels. *Sci. Agric.* **13**, 304.

Niethammer, A. (1939). Mikroskopische Bodenpilze als Begleiter in Früchten und Samen. *Arch. Mikrobiol.* **10**, 13.

Oxley, T. A. & Jones, J. D. (1944). Apparent respiration of wheat grains and its relation to a fungal mycelium beneath the epidermis. *Nature, Lond.*, **154**, 826.

Peklo, J. (1913). Über die Zusammensetzung der sogenannten Aleuronschicht. *Ber. dtsch. bot. Ges.* **31**, 370.

Sampson, K. (1937). Further observations on the systemic infection of *Lolium*. *Trans. Brit. mycol. Soc.* **21**, 84.

Schwartz-Kraepelin, H. (1947). Über das Vorkommen eines Pilzhäutchens in den Samen. *Z. Naturforsch.* **2***b*, 450.

Whitehead, M. D., Thirumalachar, M. J. & Dickson, J. G. (1948). *Microascus trigonosporus* from cereal and legume seeds. *Phytopathology*, **38**, 968.

EXPLANATION OF PLATE 8

Fig. 1. Typical subepidermal mycelium with relatively few cross-walls.
Fig. 2. Typical subepidermal mycelium with richly septate hyphae.
Fig. 3. Dark subepidermal mycelium with dark-brown cell walls.
Fig. 4. Hyphae of subepidermal mycelium associating to form a plate-like structure.

(All × 170 approx.)

(*Received* 13 *November* 1950)

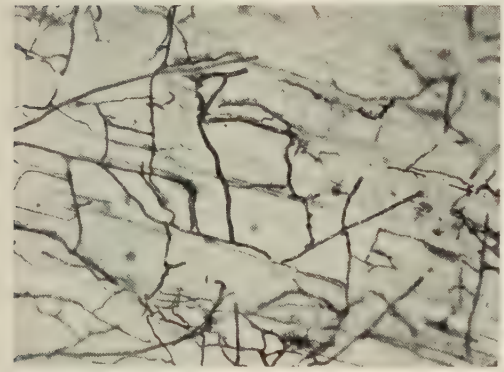


Fig. 1.

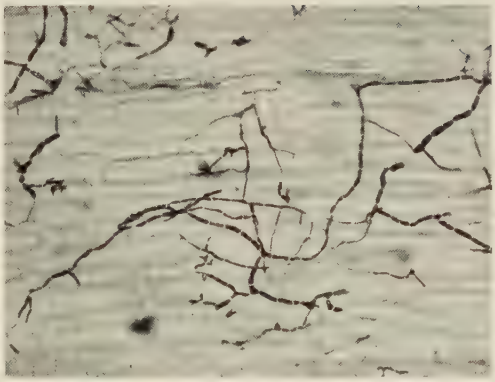


Fig. 2.

Fig. 3.

Fig. 4.

DEPOSITION OF AIR-BORNE *LYCOPODIUM* SPORES ON CYLINDERS

By P. H. GREGORY

Rothamsted Experimental Station, Harpenden, Herts

(With Plate 9 and 7 Text-figures)

For the study of deposition of air-borne particles on plant and trap surfaces a small wind tunnel has been constructed giving turbulent or streamline flow up to about 10 m./sec. The efficiency with which cylinders of from 0·018 to 2·0 cm. diameter, coated with glycerine jelly, catch *Lycopodium* spores at wind speeds over the range 1–10 m./sec. has been measured experimentally with a Cascade Impactor, operated iso-kinetically, as standard. Efficiency has been found to increase as wind speed increases, and as cylinder diameter decreases. Similar effects have been observed in the field. Efficiencies observed are lower than predicted by Sell and Glauert, but agree well with those predicted by Langmuir and Blodgett, except with the narrowest cylinders. The standard vertical sticky traps used in routine trappings of fungus spores, pollen, and crop-protectant sprays and dusts have a low trapping efficiency.

The relation between the number of spores deposited per unit area of leaf surface from a wind carrying a given number of spores per unit volume is an unstudied problem in plant pathology (Gregory, 1945). Its elucidation is a matter of general interest in biology, and is also relevant to applying protectant dusts and sprays to crops. Study of the factors controlling deposition requires experiments in the open air, and under controlled conditions in a wind tunnel. Before attempting to study deposition on plant surfaces with complicated textures, such as leaves and stems, the rules of deposition will have to be worked out for smooth geometrical bodies such as circular cylinders and plane surfaces. The mode of action even of the more or less standard sticky glass plates and cylinders used in routine spore and pollen trapping has received little attention. This paper describes experiments carried out in a small wind tunnel on the deposition of spores of *Lycopodium clavatum* on sticky cylinders of the type used for pollen trapping by Rempe (1937) at Göttingen. It is intended to report on the behaviour of spores of other sizes and on other types of trap surface in due course.

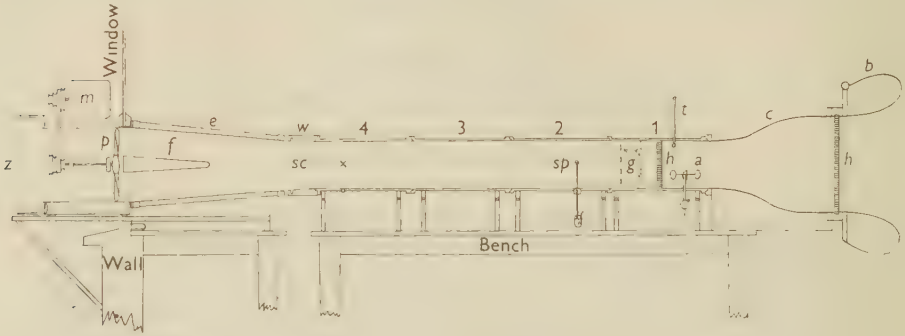
Methods

The wind tunnel

A straight-through suction wind tunnel with 1 ft. square cross-section, designed to work at wind speeds of up to 10 m./sec., was built for the investigation (Text-fig. 1).

The bell-shaped intake (*b*) and contraction (*c*) were made of cardboard stretched over plywood formers, to a design supplied by the Aerodynamics Division of the

National Physical Laboratory. The throat of the intake contains a straightener (*h*) 2 ft. square by 1½ in. thick consisting of paper honeycomb (dwarf mesh 'Dufaylite': Dufay-Chromex Ltd.).



Text-fig. 1. Diagram of wind tunnel in vertical axial section (explanation in text).

The four working sections, each 2 ft. long with an 11½ in. internal cross-section, are built of ⅜ in. transparent acrylic resin sheet (Perspex), and are arranged on adjustable supports 10 in. above a working bench. Before use the Perspex surfaces are treated with 'Cirrasol' (I.C.I.) to remove static charge. Section 1 is fitted for measuring and conditioning the air flow. It carries a Sheppard cup anemometer (*a*), standard wet- and dry-bulb thermometers (*t*), and a thermistor anemometer (Penman & Long, 1949) for measuring very low wind speeds. Another square of paper honeycomb is inserted in the middle of this section to remove the shadows of the instruments. The down wind half of the section is fitted with removable grids (*g*) to generate eddies when turbulent conditions are required. The grids are 6 in. apart and each consists of four strips of brass, 1 in. wide by 11 in. long, presenting a bluff surface to the wind. The strips are arranged vertically in the first grid, horizontally in the second (Pl. 9, Fig. 1).

Section 2 is for the spore input mechanism (*sp*) and is fitted with interchangeable top and bottom Perspex panels, drilled with holes of various size according to the requirements of the experiment (p. 360).

Sections 3 and 4 are for the diffusion of the spore cloud and for trapping. In the series of experiments described here trapping was carried out at a point (*x*) on the central axis of the tunnel, 1·4 m. from the point source in the input section.

The working sections are followed by a short section consisting of a wooden box (*w*), made readily removable to give access to equipment in the trapping section. The box carries a copper gauze screen (*sc*) to equalize suction from the fan over the cross-section of the tunnel. When the working sections are streamlined the grids are removed from Section 1, and placed in this box to keep the total resistance and wind speed in the tunnel the same as when turbulent.

This expansion is separated from the working sections by a ¾ in. air gap, to prevent

vibration from the fan from being transmitted up the tunnel. The gap is normally closed by a canvas strip. The expansion (*e*) is made of plywood, and has the function of converting the cross-section of the tunnel from an 11½ in. square to an 18 in. circle, by means of bent triangular pieces of plywood let into the corners. The expansion supports a stationary faring (*f*) for the propeller, and is attached to the metal duct housing the fan through a sponge rubber gasket.

The fan (*p*), supplied by the Airscrew Company Ltd., Weybridge, is a 4-bladed wooden propeller in an 18 in. duct, absorbing 0·56 h.p. at 2850 r.p.m. The fan is driven by a V-belt and pulleys from a ¾ h.p. squirrel cage motor (*m*), running at constant speed. The fan speed is controlled by a series of four pulleys. With the tunnel as normally fitted the pulleys give speeds of 9·8, 5·75, 3·3 and 1·75 m./sec. A speed of 1·15 m./sec. is obtained by omitting the canvas coupling with the expansion, and inserting a perforated zinc screen in the wooden box. Lower speeds are obtained by adding fabric screens.

When in use the fan projects from the window of the room in which the tunnel is housed, and its end is covered with a perforated zinc screen (*z*) to reduce the effects of external wind. Experience shows that the tunnel is erratic when wind speeds outside the building exceed about 3 m./sec. When in use the joints between sections, and all similar apertures, are sealed with transparent adhesive tape.

Wind-velocity profile

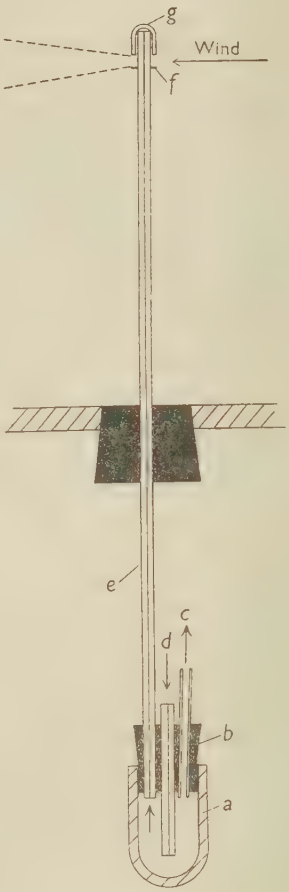
The air in a very thin layer in contact with the walls of the tunnel is at rest, and the velocity cannot, therefore, be uniform across the tunnel. The profile was investigated by probing with a form of pressure plate anemometer (the Metro-Vic 'Velometer') in the spore input position (Section 1), taking readings every half an inch across the tunnel. This instrument indicated that with a wind speed of approximately 10 m./sec. on the axis of the tunnel, the velocity was 10–12% higher at a point about 1½ in. from the wall. At distances of 2 in. from the wall and over the variation in different positions was 5–6%. In Section 4 the variation in wind speed across the tunnel was about 3%. Because of these variations all tests were done as near the axis of the tunnel as possible.

Spore input mechanism

The spores are liberated from approximately a point source on the axis of the tunnel in Section 2. The apparatus evolved for this purpose after empirical tests is illustrated in Text-fig. 2. About 20 mg. of *Lycopodium* spores are placed in a Perspex bottle (*a*) and weighed to within 0·2 mg. The bottle is then placed on the rubber stopper (*b*), which carries a simple manometer (*c*), and a centrally placed 1 mm. bore capillary tube (*d*) with the end ground flat and reaching nearly to the curved bottom of the bottle. This tube is connected to a compressed air supply.

At one side the rubber stopper also carries the spore delivery tube (*e*) consisting of a length of capillary tube, 1 mm. bore, reaching to the centre of the tunnel.

The delivery tube is fitted with a brass ring, 1 cm. diameter (*f*) 1·4 cm. below the top, and a loose-fitting glass or brass thimble (*g*) rests on the top of the delivery tube, which is itself ground flat. A cylindrical space 0·5 cm. wide is left between the bottom of the thimble and the brass ring to allow the escape of spores. When the ejector is in use the compressed air supply is adjusted, to give a pressure of 30 cm. of water in the manometer, forcing the spore cloud to rise up the capillary delivery tube to escape under the thimble. To prevent the spores sticking, the bottle is vibrated by a relay for alternate half seconds. The spore cloud so obtained is not uniform in time but decreases exponentially. For the purpose of the investigation this does not matter, as calculations are based on the total number of spores passing through a cross-section of unit area.



Text-fig. 2. Spore input mechanism as used for *Lycopodium* (explanation in text).

Traps

The trap under test is inserted in Section 4. In the series reported here the traps consisted of glass cylinders of various size, placed vertically across the axis of the tunnel. Diameters tested covered the range 0·02–2·0 cm. The wider cylinders were 11½ in. long and reached from top to bottom of the tunnel. Cylinders less than 0·1 cm. diameter were shorter and were held erect in the centre of the tunnel by fixing to a stout glass rod.

After a test of various adhesives, glycerine jelly (gelatine 1 g., glycerine 7 g., water 6 c.c., phenol 1 %) was adopted. With cylinders of diameter 0·5 cm. and larger the adhesive was applied by dipping a strip of transparent cellulose film into molten jelly, draining and winding round the cylinder. After exposure (with the overlap of the strip placed downwind) the film was stripped off and mounted on a slide for counting. Narrower cylinders were coated with the jelly direct on the glass, and after exposure were counted by vertical illumination on the microscope stage. Other adhesives caught fewer spores than glycerine jelly, or were less

convenient to handle. The relative catch, taking glycerine jelly as 100%, in a small number of tests under standard conditions was: vaseline, 90%; tree-banding grease, 30%; and transparent adhesive tape (probably coated with poly-isobutene), 14%. 'Cellofass B' (I.C.I.), the sodium salt of a cellulosic acid, in 5% aqueous solution with 5% glycerine was as efficient as glycerine jelly, but less easy to apply to the trap.

The deposit on the trap was measured by direct counting of particles under the microscope using a graticule in the ocular and making ten traverses round each cylinder.

Lycopodium *Spores*

The spores of *Lycopodium* are particularly suitable for experimental work because, as noted by Zeleny & McKeehan (1910), they separate readily into single units in air. A single commercial sample of *Lycopodium* (B.D.H.) was used in all the experiments. The characteristics of *L. clavatum* have been determined by various workers, as follows, including determinations made during this study:

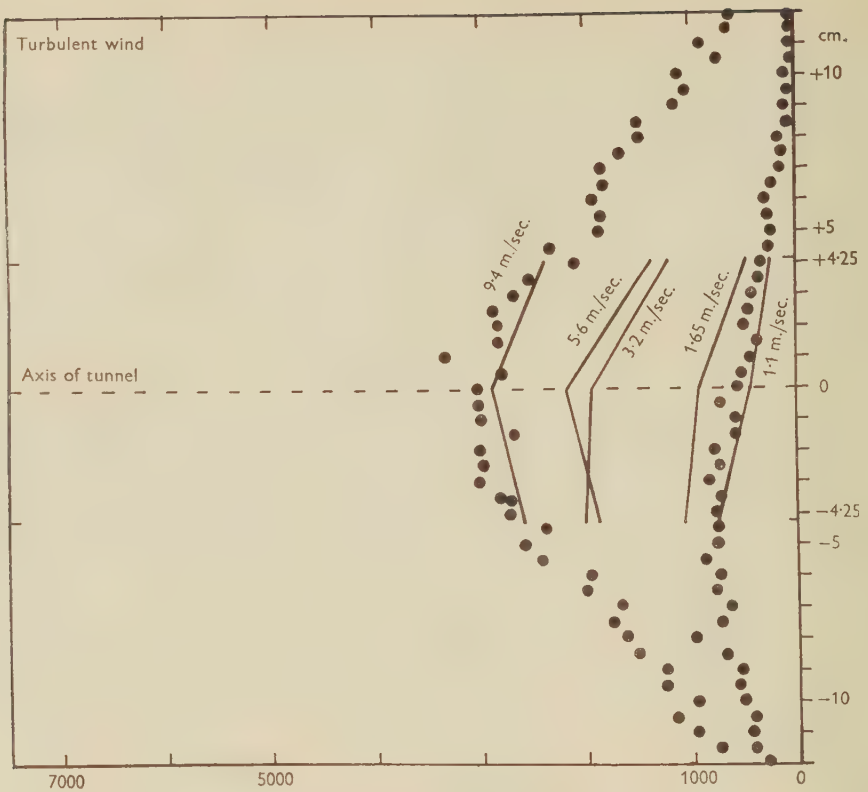
Observer	Diameter (μ)	Density	No. per g.	Observed terminal velocity (cm./sec.)
Zeleny & McKeehan (1910)	31·6($\pm$2)	1·175	—	1·76
Wallis (1919)	—	—	$9{\cdot}4 \times 10^7$	—
Stepanov (1935)	34·0	—	$4{\cdot}05 \times 10^4$(?)	2·14
Gregory	32·2 $\pm$ 1·8 (distal view)	—	$9{\cdot}39 \times 10^7$	—

The speed of fall expected from Stokes's law is 3·6 cm. sec. The values adopted here are those of Zeleny & McKeehan for terminal velocity, and of this laboratory for diameter and number per g.

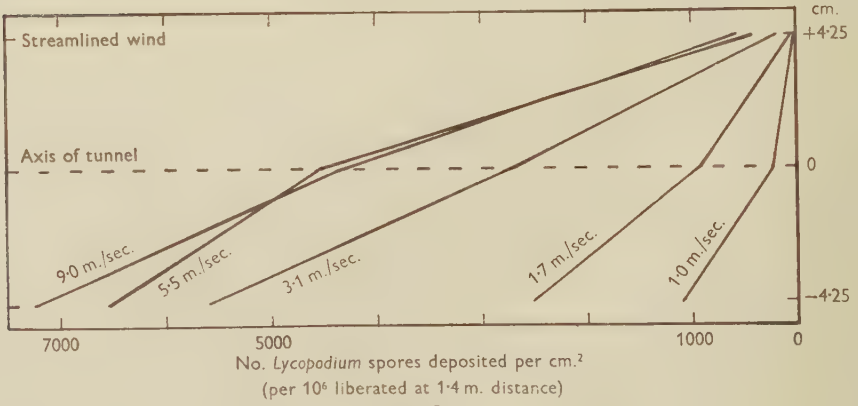
Cross-wind variations in spore deposition

With the point-source only 1·4 m. upwind, a uniform spore density across the tunnel would not be expected at the trapping position even under turbulent conditions. The actual deposition of spores in different positions across the tunnel was studied by coating a glass cylinder, 0·53 cm. diameter, and recording the density of deposit in the trapping position every $\frac{1}{2}$ cm. from top to bottom of the tunnel at a high and a low wind speed, and at three fixed positions for five wind speeds under turbulent and streamline conditions (Text-figs. 3, 4).

In turbulent wind the region of greatest density is axial at 9·8 m. sec., and about 6 cm. below the axis at 1·1 m./sec. (The deposit per unit area in the region of greatest density also decreases at low wind speeds because, as shown below, the trapping efficiency of a cylinder is low at low wind speeds.) In streamlined wind the regions of greatest density are evidently below the lowest point sampled. At the highest wind speed the cloud is denser in streamlined than in turbulent air.



Text-fig. 3.



Text-fig. 4.

Text-figs. 3 and 4. Vertical cross-wind distribution of *Lycopodium* spore cloud as shown by deposition on cylinders, 0·53 cm. diameter. Fig. 3. Turbulent wind. Data for points on axis and at +4·25 and −4·25 cm. for five wind speeds, and every 0·5 cm. from +12 to −12 cm. for 9·4 and 1·1 m./sec. Fig. 4. Stream-lined wind. Data for points on axis and at +4·25 and −4·25 cm. for five wind speeds.

Although large variations in spore deposition occur in different positions across the tunnel, even at 1·4 m. from the source, it is clear that the axis of the tunnel is a region of relatively slight change in density with height. Early attempts to compare two traps simultaneously were therefore abandoned in favour of using a single trap placed on the axis in each run.

Distribution of spores on trap surface

The distribution of spores on the trap surface would be expected to follow the Poisson distribution, if the particles in fact travelled and were deposited independently. Four cylinder traps, 0·53 cm. diameter, were exposed to spore clouds in a wind of 5·9 m./sec., and the densest part of the trace (the stagnation line) examined under a graticule and the number of spores counted square by square (occasional large clumps of spores were disregarded in making the counts). The density of deposit was arranged so that most squares contained 0 or 1 spore. Comparison of observed and expected frequencies gave $\chi^2 = 3{\cdot}272$ for $n = 7$, which corresponds to a probability between 0·8 and 0·9, showing good agreement with the Poisson distribution. A mean count of 100, based on the usual ten traverses, would therefore have a standard error of 3·16.

Method of investigation

The presence of a cylinder, or other solid body, in an air stream causes deflexion of the approaching streamlines (Sell, 1931). Some of the airborne particles which approach are also deflected while others, because of their momentum, are impacted on the surface. The efficiency, E, of the cylinder in trapping particles, is defined as the number of particles deposited on unit cross-wind presentation area of trap, expressed as a fraction of the total number of particles whose trajectories in the undeflected column of air would have passed through that area. (More simply: trapping efficiency is the deposit on the trap as a percentage of the spores that would have passed through the space occupied by the trap, had it not been there). 'Trapping efficiency' of a surface trap must not be confused with 'impaction efficiency' of May (1945), and 'collection efficiency' of Bourdillon, Lidwell & Lovelock (1948), which are properties of suction traps.

To determine the efficiency of a trap experimentally under a given set of conditions two quantities must be measured: (1) the number of particles passing through unit area of a plane at right angles to the wind; and (2) the number of spores deposited per unit presentation area of trap under the same conditions. As a spore cloud of uniform cross-wind density cannot be produced in a wind tunnel without an inconveniently long path for diffusion, it has not proved practicable to measure both quantities simultaneously in the same experiment. The two quantities have therefore been measured at the same position in the diffusion cone during successive replicated runs under each set of conditions.

Cleaning the tunnel between runs at the same wind speed was found to be unnecessary, as spores deposited on the floor or walls were not picked up by the wind. After changing from one wind speed to another, it was necessary to 'condition' the tunnel by means of a dummy run, to adjust the deposit adhering to input and other surfaces.

Measurement of spore cloud density by suction traps

The concentration of the spore cloud on the axis of the tunnel 1·4 m. from the source has been estimated by means of the Cascade Impactor. The operation of this instrument, and general principles of sampling aerosols by suction traps, have been described by May (1945). The instrument consists of a folded tube through which the air to be sampled is drawn. During its passage the air is accelerated through a series of four orifices and the particles are impacted on sticky slides placed behind the orifices. The first orifice faces the wind and the rate of flow through the instrument is adjusted so that the velocity of the air entering the first orifice is the same as the wind being sampled (isokinetic sampling). Air was drawn through the instrument by means of a vacuum pump (W. Edwards, Type IV), and the rate of flow measured by a 'Rotameter' or by an orifice plate flowmeter. From the numbers of particles deposited on the various slides the number in the volume of air sampled can be ascertained. (The small correction to allow for loss on the impactor walls was found to vary from about 1% when sampling at 50 l./min. to 4% at 6 l./min.)

The time-mean density of the cloud in the standard sampling position was estimated under turbulent and streamline conditions for each of the standard wind speeds.

Table 1 is based on a number of runs at each wind speed and shows in columns 6 and 7 the number of spores passing through an imaginary area of 1 cm.² normal to the axis (Landahl & Herrmann (1949) give this the convenient name of 'area dose'). A few tests with a suction trap of the Pasteur (1861) type, consisting of a disk of Whatman's no. 4 filter-paper inserted across a brass tube 1 cm. diameter with a feathered orifice gave values similar to the Cascade Impactor.

TABLE 1. *Time-mean concentration of* Lycopodium *spores in air at a point on axis of wind-tunnel, 1·4 m. from point source*

Wind speed (m./sec.)		Rate of sampling with Cascade Impactor (l./min.)	Mean no. of spores per m.³ per million liberated per min.		No. spores passing through 1 cm.² normal to axis	
Turbulent	Streamline		Turbulent	Streamline	Turbulent	Streamline
(1)	(2)	(3)	(4)	(5)	(6)	(7)
9·70	9·38	50	76,020	139,800	4500	7870
5·75	5·55	30	126,600	256,800	4420	8550
3·30	3·17	16·5	190,600	436,900	3810	8310
1·75	1·68	9	299,100	378,400	3210	3810
1·15	1·07	6	459,600	242,900	3190	1560

In turbulent wind the concentration on the axis is, as would be expected, approximately inversely proportional to the wind speed, because the million spores liberated each minute are suspended in greater volumes of air at the higher wind speed. Deviations from this rule can be accounted for by the downward displacement of the region of maximum density of the dispersal cone, shown in Text-fig. 3. Sutton's hypothesis that to a first approximation, dilution of a cloud by eddies during travel is independent of wind speed is confirmed under the conditions of the test.

Under streamline conditions the densities on the axis are generally higher than when turbulent. The decrease in density with decreasing wind speed is very marked and is again probably due to the downward displacement of the centre of the cloud under gravity.

Impaction on cylinders

Efficiency

Deposition on circular glass cylinders of various diameters coated with glycerine jelly placed vertically in the centre of the tunnel 1·4 m. from the source, was measured at five wind speeds. The results are expressed in Table 2 as the number of spores deposited per cm.^{2} presentation area of the trap (diameter × length), so that the efficiency of impaction can be calculated directly from column 6 of Table 1. To obtain the mean density of deposit per cm.^{2} on the actual upwind half-surface of the cylinder, the values given in Table 1 should be multiplied by $\frac{1}{2}\pi$.

Table 2. *Number of* Lycopodium *spores deposited per cm.*^{2} *(presentation area) per million spores liberated on axis of wind tunnel (turbulent) 1·4 m. from point source*

Cylinder diameter (with adhesive) (cm.)	*Wind speed* (m./sec.)				
	9·7	5·57	3·3	1·75	1·1
2·0	1200	807	429	75	8
1·2	1700	1330	650	231	57
0·84	2250	1840	1340	562	245
0·65	2340	2030	1650	909	422
0·53	2580	2180	1750	907	555
0·38	2820	2700	2050	1400	880
0·32	2830	2900	2440	1390	1050
0·22	3060	3079	2650	1900	1370
0·08	3520	3520	3030	2640	2320
0·018	3430	3660	3320	2800	3010

Entries in Table 2 are means of from three to twelve runs. The greatest number of tests at each wind speed was done with the 0·53 cm. cylinder. This size is convenient for field work, and it was necessary to know its efficiency with greater precision.

The trapping efficiency, E, for each cylinder at each wind speed can be calculated

by dividing the individual entries in Table 2 by the values, at the appropriate wind speed, in Table 1, column 6. Alternatively the data in Table 2 could be used to estimate the number of spores per m.^{3} in the column of air sweeping the trap surface each minute, and expressing this as percentage of the approximate entry in Table 1, column 4. The values for E so obtained are given in Table 3. The data in Tables 2 and 3 refer to turbulent conditions.

TABLE 3. *Efficiency of trapping on cylinders in turbulent wind* (E%).

Cylinder diameter (with adhesive) (cm.)	*Wind speed* (m./sec.)				
	9·7	5·75	3·3	1·75	1·1
2·0	26·7	18·2	11·2	2·33	0·25
1·2	37·8	30·8	17·0	7·2	1·78
0·84	50·0	41·6	35·1	17·5	7·67
0·65	52·0	45·9	43·4	28·2	13·2
0·53	57·4	49·8	46·0	28·2	17·4
0·38	62·6	61·0	53·9	43·6	27·6
0·32	63·0	65·6	64·0	43·3	32·9
0·22	68·0	69·4	69·6	59·2	43·0
0·08	78·2	79·6	79·5	82·2	72·6
0·018	76·2	82·8	87·2	87·2	94·4

For some purposes the number of particles deposited on unit *length* of the cylinder is of more interest than either the number deposited on unit *area*, or the efficiency of deposition. The observed deposit of *Lycopodium* per centimetre length of cylinder under the conditions tested is shown in Table 4. At the two higher wind speeds the total deposit is greater on the wider cylinders. At low wind speeds however there is a maximum deposit on cylinders of intermediate size, and the increased area of the wider cylinders does not compensate for their greatly reduced trapping efficiency. For routine spore or pollen trapping wide cylinders evidently have two disadvantages: not only has the deposit to be searched for over a larger area, but the total catch may be less.

TABLE 4. *Deposit of Lycopodium spores on* 1 cm. *length of cylinder*

Cylinder diameter (with adhesive) (cm.)	Wind speed (m./sec.)				
	9·7	5·75	3·3	1·75	1·1
2·0	2400	1614	858	150	16
1·2	2040	1590	780	278	68
0·84	1890	1545	1135	471	205
0·65	1520	1320	1075	590	274
0·53	1360	1155	928	482	294
0·38	1070	1030	780	532	334
0·32	908	930	783	445	336
0·22	672	675	552	418	305
0·08	282	282	243	212	186
0·018	62	66	60	50	54

Isokinetic sampling with the impactor is essential at the higher wind speeds. In a series of tests reducing the sampling rate by one-fifth below the isokinetic rate led to underestimation of cylinder efficiency by 20% at 9·7 m. sec. and 10% at 5·75 m./sec., but produced no change at 1·1 m./sec.

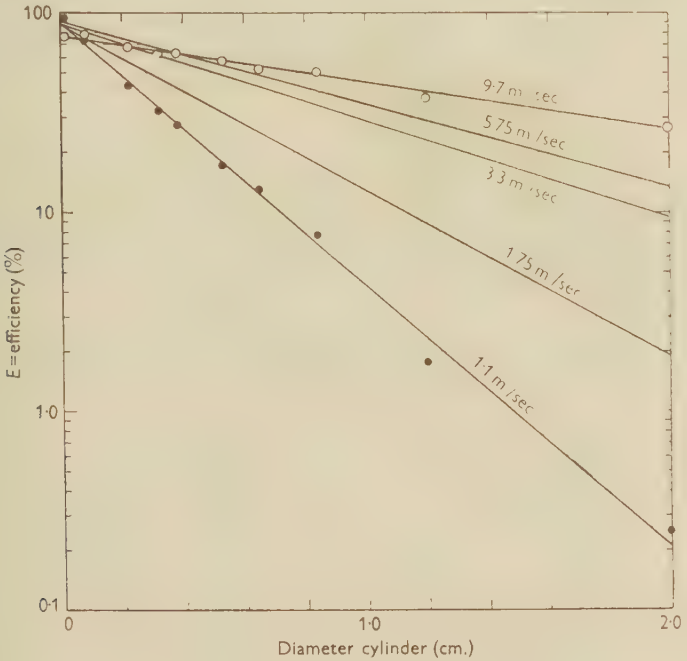
Examination of the data in Table 3 disclosed an approximately straight-line relationship between the cylinder diameter and log E. The linear regression was calculated and coefficients for the equation log $E = a + (b \times$ diameter) are shown in Table 5.

TABLE 5. *Coefficients of linear regression of log E on cylinder diameter*

(log $E = a + (b \times$ diameter) (cm.))

	Wind speed (m./sec.)				
	9·7	5·75	3·3	1·75	1·1
a	1·74	1·70	1·63	1·43	1·13
b	−0·237	−0·411	−0·475	−0·814	−1·303

The curves so fitted are shown in Text-fig. 5. The experimental values from which the lines for 9·7 and 1·1 m. sec. are derived are also shown in the diagram (other experimental values are omitted for sake of clarity).



Text-fig. 5. Linear regression of log E on cylinder diameter at five wind speeds (experimental values shown for 9·7 and 1·1 m./sec. only).

Efficiency increases as wind speed is increased, and as diameter of cylinder is diminished. There is an obvious flattening of the curve for the narrowest cylinders at the higher wind speeds. The result of this is that with the 0·08 and 0·018 cm. cylinders efficiency apparently falls slightly as wind speed is raised.

Distribution of particles over surface of cylinder

The previous section deals with mean density of deposit over the upwind half circumference of the cylinder. (It had been found that the downwind deposit was nil except for small deposits on the back of narrow cylinders at very low wind speeds). The deposit was not uniform over the surface, being densest on the mid-line of the cylinder facing the wind (stagnation line), tapering off towards the sides, and with a spore-free zone on the shoulder of the cylinder tangential to the wind direction. The width of the deposit (trace) was less than 180° in both turbulent and streamline air. The distribution in various parts of the trace was investigated to find how far changes in efficiency were caused by: (1) width of trace; and (2) density of trace.

The trace width on the cellulose film was measured on the vernier of the microscope stage. Representative values are given in Table 6. Increase of wind speed increases the width of the trace, and increase of cylinder diameter decreases it.

TABLE 6. *Angle of trace in relation to wind speed and cylinder diameter*

	Diameter (cm.)	Wind speed (m./sec.) 9·7	5·75	3·3	1·75	1·1
Turbulent	2·0	68°	52°	42°	25°	20°
	1·2	67°	60°	50°	36°	25°
	0·85	69°	67°	53°	42°	33°
	0·65	77°	64°	56°	47°	37°
	0·54	77°	67°	61°	51°	43°
Streamline	0·54	70°	62°	60°	40°	32°

The density of the trace has been averaged over 10° intervals for a number of typical individual runs. Examples based on single experiments are given in Table 7, expressed as percentage efficiency in the various positions.

TABLE 7. *Efficiency of trapping* Lycopodium *in different parts of a cylinder*

	Wind speed: 9·7 m./sec.		1·1 m./sec.	
Cylinder diameter ...	0·53 cm.	1·2 cm.	0·53 cm.	1·2 cm.
Angle	%	%	%	%
0°–10°	67	40	32	3·6
10°–20°	60	37	31	1·7
20°–30°	59	38	26	0·76
30°–40°	58	33	19	0·06
40°–50°	57	23	12	0
50°–60°	49	19	3·3	0
60°–70°	36	6	0	0
70°–80°	24	0	0	0
80°–90°	3	0	0	0
Mean *E*, for whole cylinder (Table 3)	58·1	38·4	17·5	

Comparison of Tables 6 and 7 shows that the low efficiency associated with wide cylinders and slow winds is due both to the trace being narrower and the deposit less per unit area.

Orientation

The spores of *Lycopodium*, although treated in most calculations as spheres, are in fact nearly tetrahedra, with one face rounded and three faces flattened as the result of mutual pressure with other members of the tetrad.

Spores may be deposited on the trace with any orientation but in different regions of the trace different orientations predominate. At the centre of the trace in the region of the stagnation line, most spores have the point sticking into the adhesive and have the domed distal surface exposed. At the margins of the trace the spores tend to lie on their sides, the distal surface towards the centre of the trace and the points directed towards the shoulder of the trap. This can be most simply explained on the hypothesis that the spores travel point first when they have motion relative to air. This was supported by the observation that *Lycopodium* spores landed point downwards when allowed to sediment under gravity through a column of still air on to a horizontal slide. While being transported by wind *Lycopodium* spores probably travel pointing forwards as long as their only movement relative to the suspending medium is due to gravity and drag. When they approach a cylinder or other obstacle and the streamlines diverge, the spores, temporarily retaining their original direction of travel, probably become oriented instantly into the new position.

Relevance to field conditions

Conditions in the wind tunnel, although under fairly precise control, are to some extent unnatural. In the open air the range of eddy size is much greater, and thermal effects are often pronounced. Caution is therefore necessary in extrapolating to field condition. However, some of the phenomena described above were already known to me qualitatively, from field observation before wind tunnel experiments started.

Table 8. *Vertical cylinders exposed at height* 24·4 *m. above a grass field at Rothamsted,* 2 *July* 1948, *for* 7 *hr. in wind averaging* 1·8 *m./sec.*

	Grass pollens		*Erysiphe* conidia	
	No. per traverse around cylinder 1 mm. high	No. per cm.²	No. per traverse around cylinder 1 mm. high	No. per cm.²
Cylinder diameter: (cm.)				
12·5	103	31	14	4
7·3	117	56	7	3
3·3	112	129	10	12
1·5	119	274	26	60
1·1	83	276	12	40
0·75	84	420	29	145
0·25	44	564	18	230
Horizontal slide:	—	78	—	10

TABLE 9. *Vertical cylinders exposed at two heights above a grass field at Rothamsted, 28 July 1948, for 7 hr.*

(Number deposited per cm.²)

Height (m.) ...	0·2					24·4				
Wind (mean) (m./sec.) ...	1·25					2·73				
Cylinder diameter (cm.)	Grass pollens	*Helminthosporium*	*Erysiphe*	*Alternaria*	*Cladosporium*	Grasses	*Helminthosporium*	*Erysiphe*	*Alternaria*	*Cladosporium*
3·3	17	0·6	0·3	0·3	3·9	52	2·5	13	19	447
1·5	37	1·2	3·7	0	34	83	8	63	69	51
1·1	38	2·5	0·8	6·7	23	44	13	65	110	646
0·75	36	3·7	18	7·4	136	68	10	91	136	576
0·45	133	12·0	62	24	339	82	20	182	223	4400
0·25	159	11·0	111	141	3770	141	18	248	444	5000
Horizontal slide	40	3·0	4·0	87	136	20	4·0	9·4	8·3	200

The decrease of efficiency with increasing cylinder size was first noticed in the field. Vertical cylinders of various diameter, coated with glycerine jelly on cellulose film, were exposed from a lattice tower on 2 July 1948 over a grass field. In this experiment 95% of the pollen and 92% of the *Erysiphe* conidia were on the windward (W.S.W.) side of the cylinder, thus agreeing well with the restricted position of the trace observed in wind-tunnel experiments.

Similar data were obtained by trapping simultaneously close to the ground and also at 24·4 m. in the lattice tower on 28 July 1948 (Table 9).

DISCUSSION

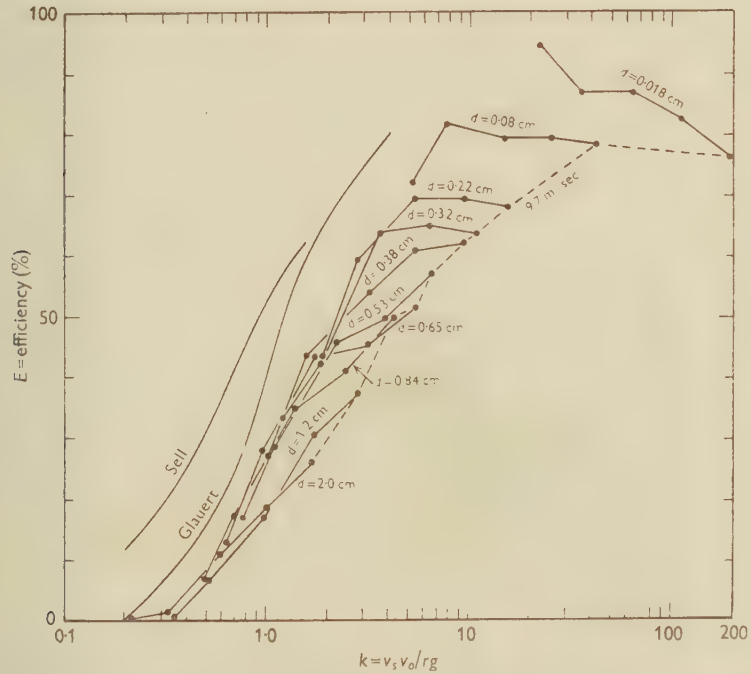
The term 'deposition' is used here in a general sense to include all processes by which air-borne particles or droplets are transferred from suspension in a fluid to the surface of a solid or liquid. In the deposition of fungus spores two main effects can readily be distinguished: (1) 'impaction' when the movement towards the surface results from the momentum of the wind-blown particle; and (2) 'sedimentation' due to motion at the terminal velocity under gravity.

The deposition of spores on a vertical cylinder or plate placed across a wind stream is mainly due to impaction. For liquid droplets the general principles of impaction have been made clear by Sell (1931) in connexion with dust filtration. Sell showed that when a bluff object was placed in the path of an air-borne particle, the particle continued to travel towards the object for some distance after the air in which it was suspended had become deflected to by-pass the object. He showed that the distance travelled by the particle, before in turn being deflected from striking the object, is related to its speed of fall under gravity in still air, as well as to the wind speed and the size of the object disturbing the flow. Sell further showed from aero-dynamical considerations that what we have defined as the efficiency of trapping, E, should be related to a non-dimensional constant which may be written as:

$$k = \frac{V_s V_0}{rg},$$

where v_s = speed of fall of particle, v_0 = speed of wind, r = radius of cylinder (etc.), g = acceleration due to gravity. (In Sell's paper diameter is used instead of radius.)

Sell derived the relation between E and k empirically, not by counting particles, but by observing the trajectories of uniform droplets of indian ink on paper bisecting a vertical cylinder in a small wind tunnel. It is not clear from Sell's paper what range of conditions was tested. His curves predict higher efficiencies than those observed in this investigation (Text-fig. 6).



Text-fig. 6. Relation between E and $k = (v_s v_0/rg)$ according to Sell, Glauert and wind-tunnel data (●—●—●).

Glauert (1946) attempted to calculate, from first principles, the percentage of raindrops caught on a circular cylinder. Like Sell, she obtained a dimensionless constant related to efficiency. After rearrangement, using Stokes's law to substitute for v_s, and by neglecting the correction for air density (negligible at the velocities we are concerned with), Glauert's constant is found to be identical with Sell's. For any value of k, Glauert's calculated value of E is only about half that of Sell. Values observed in the wind tunnel are lower than predicted by either Sell or Glauert (Text-fig. 6). Glauert's theory predicts that impaction is zero for $k < 0{\cdot}2$, while on Sell's theory E only reaches zero at $k = 0$.

Sampling of liquid aerosols has recently been studied theoretically and in wind-tunnel experiments by Landahl & Herrmann (1949), resulting in a modification of Sell's formula.

The wind-tunnel data and values of Sell and Glauert are shown, plotted on logarithmic paper for convenience of scale, in Text-fig. 6. Observed values for each cylinder size are joined by full lines, and values for 9·7 m./sec. by a broken line. None of the observed values fall near Sell's line. The lower values of k fall nearest Glauert's line (the logarithmic scale exaggerates discrepancies at low values of k and E). At higher wind speeds efficiencies observed are lower than predicted by Glauert, and with the narrower cylinders a speed is reached where E decreases with increase of k. This effect shows an interaction with cylinder size. With cylinders of 0·38 cm. diameter or larger, E rises steadily with k over the range tested. With narrower cylinders E begins to fall as k rises at progressively lower wind speeds, and with the 0·018 cm. cylinder, there is a complete reversal of trend, E falling as k is increased over the whole range of wind speeds. This phenomenon is not predicted by either of the theoretical treatments. It is most simply explained by assuming that Glauert's theory correctly predicts impaction on an ideal cylinder, but like Sell's, neglects the presence of the boundary layer. Retention of the particles by the surface after impaction is probably not complete under all conditions with the adhesive in use. When the wind speed is low the boundary layer of still air adhering to the cylinder would be thick enough to protect the spores from being blown off. At higher wind speeds the boundary layer becomes thin, and for each cylinder size there is a critical wind speed at which blow-off increases faster than efficiency. Further work will be needed to show whether this critical speed also depends, as might be expected, on particle size, and adhesive.

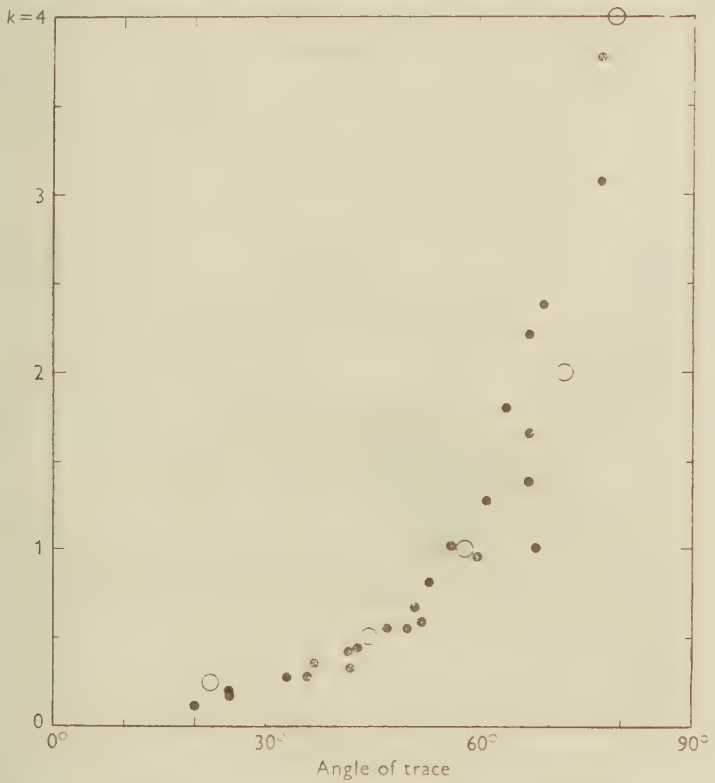
Glauert (1946, fig. 2) also satisfactorily predicted the angle occupied by the trace. Predicted and wind-tunnel values are compared in Text-figure 7.

In discussing deposition of aerosols it has been customary to use Sell's values, though Johnstone & Roberts (1949) have criticized his treatment for neglecting the boundary layer. They also state that for particles of less than 0·5 μ deposition by diffusion may exceed impaction. The wind-tunnel experiments show that in calculating the deposition of aerosols lower efficiencies will have to be assumed than those given by Sell or Glauert. Impaction on vertical plates will be dealt with in later experiments, but it is already clear that they have considerably lower trapping efficiencies than cylinders at corresponding values of k. Sell however predicted higher trapping efficiencies for flat plates than for cylinders.

Experiments have so far been restricted to *Lycopodium* spores. The general agreement of experiments with Glauert's theory, suggests that the relation between E and k (Text-fig. 6) can be assumed provisionally to hold for other particle sizes, and approximate efficiencies can therefore be read off from the graph for any particle for which v_s is known.

The data given here indicate some of the reasons for low coverage rates when crop

protectants are applied as dusts and sprays. Larger surfaces opposing the spray cone will be less efficiently covered than smaller ones; increase in the velocity of the particle or droplet will increase the efficiency of impaction. By extrapolation to other article size, it seems clear that the difficulty of covering a plant surface with spray or dust by impaction will be greatly increased with particles with v_s less than 1–2 cm./sec.



Text-fig. 7. Angle occupied by trace, as predicted by Glauert (1946) (○) and observed in wind-tunnel experiments (●).

Similar considerations apply to deposition of fungus spores on plant surfaces. The ultimate elements of feathery grass stigmas, which are of the order of 30 μ diameter probably trap grass pollen and smut spores with high efficiency. The spores of most dry air-borne leaf and stem parasites are fairly large and should be impacted under ordinary conditions at efficiencies of the order of 10%. Many typical soil inhabitants, however, are characterized by extremely small spores. The deposition by impaction of an *Aspergillus*, *Penicillium* or *Lycoperdon* spore on

a stem or leaf must be a rare event, and some other mechanism than impaction must be sought to account for their removal from the atmosphere. Such mechanisms may be sedimentation in confined spaces among vegetation, or washing of the air by rain.

The thoroughness with which particles are washed out of the atmosphere by rain becomes apparent if we assume that a raindrop traps particles with approximately the same efficiency, for a given value of k, as does a cylinder. (The values of E for spheres remain to be determined experimentally but are probably higher than for cylinders). According to data of Laws (1941) and Laws & Parsons (1944) an inch of rain at an intensity of 0·1 in. hr. would be composed largely of 1·5 mm. drops falling at a terminal velocity of about 5 m. sec. The efficiency of these droplets in sweeping up *Lycopodium* spores would correspond to $k=6$, or $E=85\%$, and for *Lycoperdon*, $E=25\%$ (Langmuir, 1948). Approximately 1500 drops of total cross-section 26 sq. cm. would fall through each imaginary plane of 1 sq. cm. cross-section at right angles to the direction of fall, so that a single *Lycoperdon* spore would be expected to have twenty-six chances of being swept up, each with an efficiency of 25%. The chance of any spore or pollen grain of the size of *Lycopodium*, or even *Lycoperdon*, remaining in the air during the fall of 1 in. of rain must be very low.

The data obtained are of use in determining the most suitable size of trap according to the purpose in view. In trying to measure the amount of deposit of insecticide or fungicide, it is advisable to use traps simulating the object being sprayed as closely as possible in size and orientation.

Table 10. *Percentage efficiencies of vertical traps in routine use*

	Wind speed (m./sec.)				
Type of trap	9·8	5·75	3·3	1·75	1·1
Vertical slide; 3 × 1 in. (76 × 26 mm.)	23	12	2·4	0·43	0·16
Cylinder; 1·4 cm. (Rempe, 1937)	36	28	16	6	2
Cylinder; 0·53 cm. (Rothamsted)	58	50	47	29	18

As a result of early field experiments described in Tables 7 and 8, the Rempe cylindrical trap 1·4 cm. diameter was replaced for routine spore trapping at Rothamsted by a cylinder approximately 0·53 cm. diameter. This can be covered conveniently by a $\frac{5}{8}$ in. square of transparent cellulose film dipped in glycerine jelly. After exposure in a shelter of the type described by Hyde & Williams (1945), the square of film is mounted in glycerine jelly and covered with a $\frac{3}{4}$ in. square cover-glass. Because this trap is more efficient a smaller area needs to be scanned than on the Rempe cylinder, and it is somewhat less sensitive to wind speed. The comparative efficiencies of the vertical traps in regular use, tested by *Lycopodium* spores, are shown in Table 10. The values for the 1·4 cm. cylinder are obtained from Text-

fig. 6 after calculating k; those for the 3×1 in. (76×26 mm.) vertical glass slide anticipate a fuller description of experiments with plane surface traps.

Thanks are due to the Agricultural Research Council for a special grant to defray the cost of the wind tunnel. For detailed plans and advice on the construction of the wind tunnel and for the loan of equipment thanks are due to the Aerodynamics Division of the National Physical Laboratory, Teddington, and to the Chemical Defence Experimental Establishment, Porton. The work of Messrs A. E. Hall and A. W. Primmett who constructed the wind tunnel in the Rothamsted workshops, and the technical assistance of Mr J. Steadman throughout the investigation, are also gratefully acknowledged.

REFERENCES

Bourdillon, R. B., Lidwell, O. M. & Lovelock, J. E. (1948). Studies in air hygiene. *Spec. Rep. Ser. med. Coun., Lond.*, no. 262. London: H.M. Stationery Office.

Glauert, Muriel (dated 1940, published 1946). A method of constructing the paths of raindrops of different diameters moving in the neighbourhood of (1) a circular cylinder (2) an aerofoil, placed in a uniform stream of air; and a determination of the rate of deposit of the drops on the surface and the percentage of drops caught. *Aeronautical Research Committee, Reports and Memoranda*, no. 2025, pp. 1–12. London: H.M. Stationery Office.

Gregory, P. H. (1945). The dispersion of air-borne spores. *Trans. Brit. mycol. Soc.* **28**, 26–72.

Hyde, H. A. & Williams, D. A. (1945). Studies in atmospheric pollen. II. Diurnal variation in the incidence of grass pollen. *New Phytol.* **44**, 83–94.

Johnstone, H. F. & Roberts, M. H. (1949). Deposition of aerosol particles from moving gas streams. *Industr. Engng. Chem.* **41**, 2417–23.

Landahl, H. D. & Herrmann, R. G. (1949). Sampling of liquid aerosols by wires, cylinders and slides, and the efficiency of impaction of the droplets. *J. Colloid Sci.* **4**, 103–36.

Langmuir, I. (1948). The production of rain by a chain reaction in cumulus clouds at temperatures above freezing. *J. Meteorol.* **5**, 175–92.

Laws, J. Otis (1941). Measurement of the fall-velocities of water-drops and rain drops. *Trans. Amer. geophys. Un.* **21**, 709–21.

Laws, J. Otis & Parsons, D. A. (1944). The relation of raindrop size to intensity. *Trans. Amer. geophys. Un.* **24**, 452–60.

May, K. R. (1945). The Cascade Impactor: an instrument for sampling coarse aerosols. *J. sci. Instrum.* **22**, 187–95.

Pasteur, L. (1861). Mémoire sur les corpuscules organisés qui existent dans l'atmosphère. *Ann. Sci. Nat.* (*Zoo.*), Ser. 4, **16**, 5–98.

Penman, H. L. & Long, J. (1949). A portable thermistor bridge for micro-meteorology among growing crops. *J. sci. Instrum.* **26**, 77–80.

Rempe, H. (1937). Untersuchungen über die Verbreitung des Blütenstaubes durch die Luftströmungen. *Planta*, **27**, 93–147.

Sell, W. (1931). Staubausscheidung an einfachen Körpern und in Luftfiltern. *Forschungsh. Ver. dtsch. Ing.* **347**, 1–22.

Stepanov, K. M. (1935). Dissemination of infective diseases of plants by air currents. *Bull. Pl. Prot. Leningr.*, Ser. 2, *Phytopathology*, no. 8, pp. 1–68.

SUTTON, O. G. (1932). A theory of eddy diffusion in the atmosphere. *Proc. Roy. Soc.* A, **135**, 143–65.

WALLIS, T. E. (1919). The use of *Lycopodium* in quantitative microscopy. *Nature, Lond.*, **103**, 455.

ZELENY, J. & MCKEEHAN, L. W. (1910). Die Endgeschwindigkeit des Falles kleiner Kugeln in Luft. *Phys. Z.* **11**, 78–93.

EXPLANATION OF PLATE 9

Fig. 1. Wind tunnel: bell intake, contraction, grids for generating turbulence in position in Section 1.

Fig. 2. Wind tunnel: Cascade Impactor in trapping position in Section 4.

(*Received* 14 *August* 1950)

Note added 30 *April* 1951

While this paper was in the Press Mr R. W. Gloyne drew my attention to the work of Langmuir & Blodgett (1949) in which the problem of deposition of droplets on a cylinder was investigated by means of the differential analyzer. Instead of a single value of efficiency for each value of k, Langmuir and Blodgett obtained a series of curves in which the relation between E and k depends on another dimensionless number, $\phi = R^2/k$, where R is the Reynolds number appropriate to the particle at the wind speed. In the wind tunnel experiments described above, ϕ varied from 30 to $2{\cdot}5 \times 10^4$. Except for six observations at extreme values of k, the points plotted on Text-fig. 6 all fall within, and are closely bounded by Langmuir and Blodgett's curves for $\phi = 10$ to $\phi = 10^4$. Only the observed values for $\phi = 100$ deviate widely from expectation, presumably because of blow-off of solid particles under conditions of the experiment.

LANGMUIR, I. & BLODGETT, KATHERINE B. (1949). Mathematical investigation of water droplet trajectories. *General Electric Research Laboratory, Report No. R.L.* 225, pp. 1–47. Schenectady.

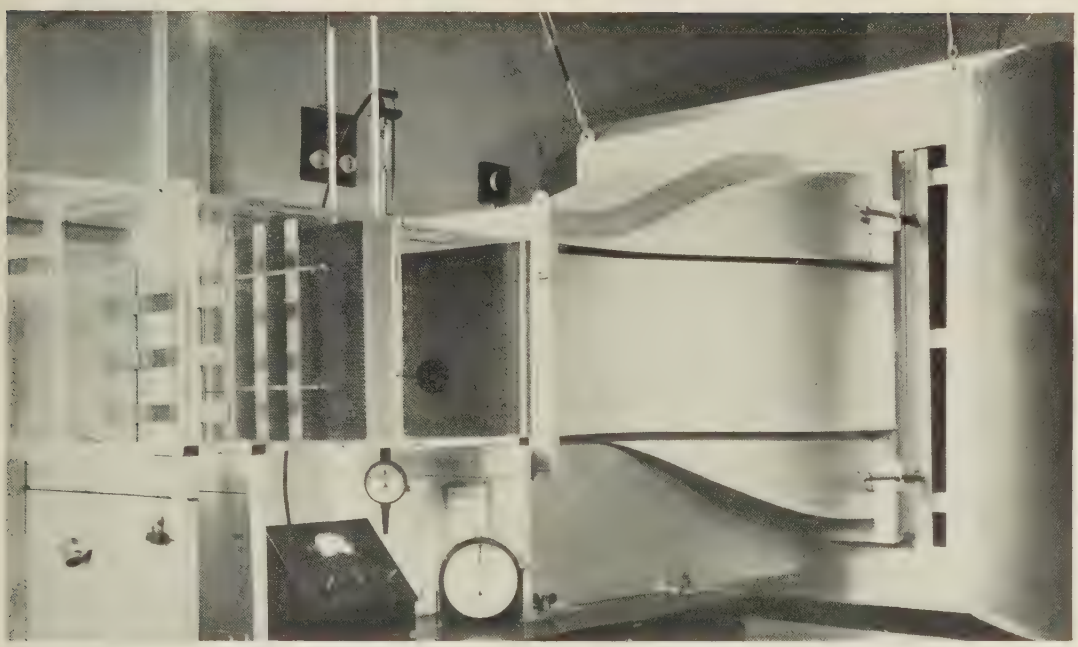


Fig. 1.

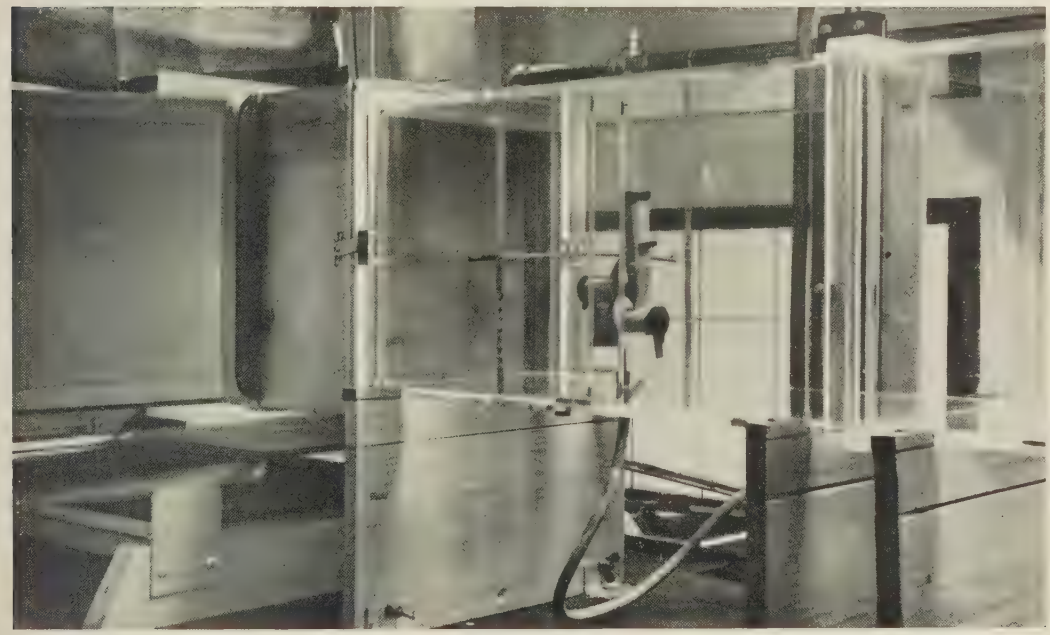


Fig. 2.

SOME PROPERTIES OF FOUR STRAINS OF CUCUMBER MOSAIC VIRUS

By K. S. BHARGAVA*

Rothamsted Experimental Station, Harpenden, Herts

(With Plate 10)

Different strains of cucumber mosaic virus differ in their host range, symptoms caused, virulence towards different plants, transmissibility by aphids, dilution end-point and thermal inactivation point.

There are seasonal variations in the susceptibility of some host species; French bean is apparently immune during the summer but during the winter produces countable local lesions suitable for quantitative assays.

Different host species differ in the ease with which cucumber mosaic virus is transmitted to and from them; systemic infection in beet rarely occurs unless the virus is introduced into young tissues. Inhibitors of infectivity in sap of sugar beet and *Phytolacca* sp. make mechanical transmission from these to other hosts difficult; the inhibitors interfere less with the infection of hosts in which they occur than with the infection of tobacco.

Cucumber mosaic virus has a low temperature coefficient of thermal inactivation and much infectivity is destroyed by heating at temperatures below the thermal inactivation point.

Myzus persicae (Sulz.) is a more efficient vector than *M. ornatus* Laing which is more efficient than *Macrosiphum euphorbiae* (Thomas); although individual aphids can cause more than one infection, most cease to be infective in feeding periods of from one to five minutes.

During the summer of 1945 a severely diseased spinach plant with chlorotic, stunted and deformed leaves was received at Rothamsted from Mr W. Buddin of Reading. The symptoms differed from any previously described, but a strain of cucumber mosaic virus was suspected to be the most probable cause. The presence of a virus was demonstrated by inoculating sap from the spinach to tobacco seedlings, which developed necrotic local lesions and a severe systemic disease. Although the symptoms differed strikingly from any previously encountered, tests for mutual antagonism between it and a range of other viruses established it to be a strain of cucumber mosaic virus. Tobacco plants infected separately with three different strains of cucumber mosaic virus were protected completely against invasion by the virus from spinach, whereas plants infected with other viruses were still susceptible. The behaviour of the virus from spinach, particularly its ability to cause countable local lesions in tobacco, seemed to make it an unusually favourable strain of cucumber mosaic virus for quantitative work. As the literature on this virus contains conflicting statements about its host range, properties *in vitro* and

* Present address: Botany Department, Allahabad University, Allahabad, India.

transmission, the behaviour of the spinach strain was studied in some detail and compared with that of three other less virulent strains. The results showed that the published discrepancies are amply accounted for by the facts that strains differ in host range and in the concentration they reach in infected plants, that different hosts differ in the ease with which the virus can be transmitted to and from them, and that susceptibility is greatly influenced by the environment.

Materials and methods

The original diseased spinach plant contained at least two strains of cucumber mosaic virus. Only one of these caused unusual symptoms, and this will be referred to as the 'spinach strain'. It was isolated from a local lesion produced in a tobacco leaf by an infective *Myzus persicae* (Pl. 10, fig. 1). The lesion was cut from the leaf, macerated and the juice from it rubbed over another leaf. The process of isolation from single lesions was repeated three times in series and the culture finally obtained produced consistent results throughout the work, except in one insect-transmission test when two plants each colonized with a single aphis produced mild symptoms.

A second strain will be referred to as the 'primula strain'; it was obtained from a naturally infected *Primula obconica* plant and has consistently produced a mild mottling of tobacco plants of the type most often recorded for cucumber mosaic virus. Two other strains were kindly supplied by Dr Kenneth M. Smith; one, which came from a naturally infected turnip, caused symptoms in tobacco similar to those caused by the primula strain, and will be referred to as the 'turnip strain'; the other was a derivative of the 'yellow strain' originally described by Price (1934), which produces a bright yellow blotchy mosaic in tobacco.

Stock cultures of the four strains were maintained in *Nicotiana glutinosa* to ensure freedom from contamination with tobacco mosaic virus. Unless otherwise stated, the inoculum used for experiments was sap from systemically infected tobacco plants (*N. tabacum*, var. White Burley). Inoculations were made by rubbing the leaves of healthy plants with the forefinger wet with inoculum; unless otherwise stated, 'celite' (a diatomaceous silica of Johns, Manville and Co.) was lightly dusted over the leaves before they were rubbed.

Aphids used for insect-transmission tests were maintained and handled by methods similar to those described by Watson (1936, 1938); the aphids were raised on healthy radish plants, from which they were either transferred directly to infected plants or were transferred after a period without food during which they were enclosed in Petri dishes with 'cellophane' covers.

Quantitative infectivity tests were made by the local-lesion method. Price (1934) found that cucumber mosaic virus produces discrete local lesions in cow pea (*Vigna sinensis*, var. Black eye), and Costa (1944) by adding carborundum to inocula used this host for quantitative work. All four strains used in the work described below produced necrotic local lesions on cow pea and all failed to become systemic, though the lesions formed by the turnip strain were less localized than those caused

by the other three and spread along the veins of the inoculated leaves. Even with the aid of carborundum or celite, however, too few lesions were obtained for quantitative assays to be reliable on this host. In the winter, local lesions tests were done with all four strains on French bean (*Phaseolus vulgaris*, var. Bountiful) and in the summer they were made only with the spinach strain in tobacco.

Host range and symptomatology

The following plants were inoculated with the four strains:

Cucumis sativus, var. Every-day. The spinach strain differed from the others in causing circular, chlorotic local lesions in cucumber. Systemic symptoms first appeared around the smaller veins of the young leaves and later changed into a general mosaic. The infected leaves were reduced in size and much distorted. Plants inoculated in the two-leaf stage were usually killed, but older plants survived and some produced a few mottled and deformed fruit. The other strains produced only systemic symptoms, similar to, but much less severe than, those caused by the spinach strain.

Citrullus vulgaris, var. Red-fleshed. None of the four strains became systemic in water-melon; the spinach strain caused water-soaked local lesions, which soon became necrotic, and the others caused chlorotic local lesions.

Nicotiana tabacum, var. White Burley. Tobacco leaves developed local lesions in 4–6 days (Pl. 10, fig. 1). Their type and severity varied with the environment and were more numerous and necrotic in winter than in summer. In winter they were at first grey and water-soaked, but soon became wholly necrotic, whereas in summer they were often predominantly chlorotic, though they sometimes had necrotic haloes. The first systemic symptoms were a clearing of the veins, which was soon followed by an intense chlorosis, distortion and curling of the young leaves, with necrosis of the veins (Pl. 10, fig. 2). Ringspot symptoms and oak-leaf patterns were also common. Infected plants temporarily ceased growth, but after a month or so there was some recovery and new leaves were produced showing a less severe mottle and distortion. It seems that the virus content of infected plants decreases with age and that the apparent recovery is correlated with a reduction in the infectivity of sap. For example, in one test comparing the infectivity of sap from plants infected for 14 days, 2, 3, 7 and 28 months, the average number of local lesions produced per French bean leaf was, respectively, 287, 105, 15, 6 and 1.

N. glutinosa. The spinach strain produced initial effects similar to those in tobacco. Local lesions, usually necrotic, were followed by a severe systemic disease. Plants infected during the winter usually died, and during the summer their growth was much reduced, while the leaves were brightly mottled and much distorted. The other three strains produced only systemic symptoms, mottles of varying intensities with leaf distortion and reduction of the laminae.

Datura stramonium. The spinach strain caused local lesions of variable types; sometimes there were large water-soaked areas that soon became necrotic, sometimes

concentric necrotic rings and sometimes small spots (Pl. 10, fig. 3). Systemic symptoms were a clearing of the veins and a brilliant mottle with necrosis and distortion of the laminae. The primula and turnip strains sometimes produced diffuse chlorotic local lesions, but usually caused only systemic symptoms, showing as a clearing of the veins, which was succeeded by a general mosaic. The yellow strain produced chlorotic local lesions, but did not become systemic.

Solanum tuberosum. Doolittle & Walker (1922) and Johnson (1927) reported infecting potato plants with cucumber mosaic virus. None of the four strains tested by the author caused infection in the varieties Arran Banner, British Queen, Craig's Defiance, Gladstone, Katahdin, King Edward and Majestic, when introduced either by sap inoculation or by aphids; nor was there any infection when Craig's Defiance, King Edward and Majestic were grafted with scions from tomato plants infected separately with the four strains.

Lycopersicum esculentum. In tomato, var. Kondine Red, the spinach strain caused necrotic local lesions and systemic symptoms of a faint mottle with curling and reduction in size of the leaf laminae. The other strains caused no local lesions but rather more severe leaf deformity. Occasionally all strains produced 'fern-leaf' symptoms, the leaves becoming filiform and consisting of little more than the main veins. Mogendorff (1930) stated that this condition was a regular result of infection by aphids but occurred only occasionally when plants were inoculated mechanically. This could not be confirmed. Tomato plants were infected at various ages, by aphids and by sap inoculation, and some were grown under glass and others in the open. Plants infected in the two-leaf stage showed more of the fern-leaf symptoms than those infected in the five-leaf stage and those infected with the primula strain showed a little more than those with other strains, but the other treatments were without effect. The fern-leaf symptoms caused by all strains were much intensified by simultaneous infection with tobacco mosaic virus, as has already been described by Limasset & Montgremier (1946) (Pl. 10, fig. 6).

Apium graveolens. Celery var. Solid White, reacted very differently to the four strains, the differences being ample to account for the conflicting statements in the literature. The spinach strain did not become systemic and the only symptom was a down-curling of the laminae of inoculated leaves. No infection was obtained with the yellow strain, whereas the primula and turnip strains became systemic and caused a general mosaic.

Phytolacca esculenta. All four strains caused somewhat similar symptoms in *P. esculenta*, diffuse chlorotic local lesions and a systemic disease, showing first as isolated chlorotic spots or rings and later as a general mosaic. As noted by previous workers (Allard, 1918; Doolittle & Walker, 1925; Price, 1940), there was difficulty in transmitting the virus from *P. esculenta* by sap inoculation, presumably because the sap contains a glycoprotein that is a strong inhibitor of infectivity (Kassanis & Kleczkowski, 1948). Of forty tobacco seedlings inoculated with undiluted sap from a systemically infected *P. esculenta* plant, only one became infected. The inhibitor

seems to have less effect in preventing infection in *P. esculenta*, for more than half the healthy plants of this species that were inoculated with sap from infected ones became infected.

Beta vulgaris. All four strains produced similar effects in sugar beet, var. Kleinwanzleben E., the only difference being that leaves inoculated with the spinach strain withered and died. Chlorotic or necrotic local lesions were followed by systemic symptoms consisting of a mosaic with dwarfing of growth and a much reduced leaf size (Pl. 10, figs. 4 and 5). Systemic infection did not always occur, and previous workers (Johnson, 1930; Samuel, 1931; Hoggan, 1933*b*; Wellman, 1935; Bennett, 1944) have usually described only local lesions, although Hoggan (1933*a*) and Wellman (1935) obtained systemic infection using infective aphids. The results recorded in Table 1 show that systemic infection depends on whether or not an abrasive is incorporated in the inoculum and on the kind of tissue rubbed; introducing the virus into young tissues favours systemic infection, which is not produced by aphids when these feed only on lower leaves.

TABLE 1. *Systemic infection of sugar beet by different modes of inoculation*

Mode of inoculation	No. systemically infected out of five inoculated with different strains			
	Spinach	Primula	Turnip	Total
(1) Rubbing outer leaves only	0	3	1	4/15
(2) Rubbing all the leaves (upper surface)	2	4	2	8/15
(3) Rubbing all the leaves (under surface)	1	4	1	6/15
(4) Rubbing all the leaves and the growing point	3	4	3	10/15
(5) Rubbing all the leaves without celite	Local	Local	Local	0/15
(6) *Myzus persicae* (restricted to centre of one leaf)	Local	Local	Untested	0/10
(7) *M. persicae* (free)	3	3	Untested	6/10

As with *Phytolacca esculenta*, cucumber mosaic virus is difficult to transmit from sugar beet to tobacco by inoculation of undiluted sap, about 20% of the plants usually becoming infected. A greater percentage, often 100, is obtained if the sugar beet sap is diluted 1/10 or 1/100. Again the inhibitor has less effect in preventing infection of the host in which it occurs than of tobacco, and transmission was regularly obtained from infected to healthy sugar beet by inoculation with the aid of celite. Sugar beet sap is a less effective inhibitor of infectivity than is *P. esculenta* sap; sap from *P. esculenta* diluted 1/10 with water when mixed with an equal volume of sap from diseased tobacco leaves completely inhibited infectivity whereas sap from sugar beet did not, though it reduced the numbers of local lesions in beans from an average of sixty per leaf to two.

Beta cicla. Spinach beet reacted to all four strains in much the same manner as sugar beet, except that systemic infection occurred more frequently. As with sugar beet, transmission of the virus from spinach beet to tobacco by inoculation was difficult.

Lathyrus odoratus. None of the four strains infected sweet pea.

Pisum sativum. All four strains produced a few necrotic lesions and a general yellowing in inoculated leaves of Lincoln peas; all also became systemic, causing a general chlorosis, and the primula strain killed infected plants within a month.

Phaseolus vulgaris. The two varieties of French bean tested, Prince and Bountiful, showed a striking seasonal variation in susceptibility. During winter, between the months of October and March, brown local lesions, either spots or rings, were regularly produced by all four virus strains in sufficient numbers for the hosts to be used satisfactorily for quantitative assays (Plate 10, fig. 7). Systemic infection never occurred, but the virus was readily recoverable from the inoculated leaves. Between the months of March and October, however, the bean plants appear to be immune; not only were no lesions produced by any of the four strains, but no virus could be recovered from the inoculated leaves.

Table 2. *Effect of periods of darkness before and after innoculation on the susceptibility of bean plants*

	Treatment of plants		
	Before inoculation (days)	After inoculation (days)	Number of lesions per 10 sq.cm.
I.	8 light	6 light	7
	8 light	6 dark	48
	8 dark	6 light	33
	8 dark	6 dark	208
II.	8 light	6 light	14
	8 light	6 dark	13
	3 light + 5 dark	6 light	31
	3 light + 5 dark	6 dark	157
III.	8 light	6 light	10
	8 light	6 dark	52
	6 light + 2 dark	6 light	21
	6 light + 2 dark	6 dark	120

Bawden & Roberts (1947, 1948) found that the susceptibility of bean plants to tobacco necrosis viruses in summer was increased by raising plants under reduced light intensity, or by keeping them in darkness for a few days immediately before they were inoculated. It seemed possible that the seasonal variation in the reaction of beans to cucumber mosaic virus might also be an effect of illumination, but experiments to test this showed that factors other than light intensity or length of day are involved. During summer, various combinations of treatments involving alterations in intensity of light, length of time each day plants were exposed to light, and keeping in darkness for various lengths of time before inoculation, all failed to produce plants that reacted with visible local lesions. Etiolation during the winter increased the numbers of local lesions produced. The results of three experiments made during January and February are recorded in Table 2. The various treatments were started as soon as the first-formed leaves were beginning to unfold. The leaves

of plants kept in the dark were more fragile, yellower and softer to the touch, than those kept in the light. The fact that periods in the dark after inoculation also increased the numbers of lesions, however, suggests that the enhanced susceptibility is not solely produced because the etiolated plants suffer more extensive injuries when rubbed. The highest numbers of lesions were always produced by plants kept in darkness both before and after inoculation, a different result from that obtained by Bawden & Roberts (1948) with tobacco necrosis viruses, with which periods in darkness after inoculation gave inconsistent results but most often reduced the numbers of lesions.

Zea mays. Systemic infection of maize by cucumber mosaic virus has been reported by Wellman (1934) and Harter (1938), whereas Price (1935) reported only local lesions. Of the four strains used in this investigation, the yellow strain caused chlorotic local lesions and systemic symptoms in the form of elongated yellow streaks, and the others produced only local lesions, starting as chlorotic spots which became necrotic as they spread along the veins of the inoculated leaves.

Dilution end-point and thermal inactivation

Different workers have recorded dilution end-points for cucumber mosaic virus varying between 1/100 and 1/10,000 and thermal inactivation points between 60 and 75° C. From what has been said above about the differences in the ease with which the virus can be transmitted to and from different host species, and the apparent fall in virus content with increasing age of infected tobacco plants, these discrepancies are partly accountable by different workers using different test plants. The results given in Tables 3 and 4 show that the dilution end-point also depends on the strain of the virus and on the method of inoculation. Inocula for these tests were all from tobacco plants that had been infected for 3 weeks and were showing advanced symptoms. Table 3 compares the infectivity of the four strains at various dilutions for their ability to produce systemic infection in tobacco plants and local lesions in Bountiful beans; in this test celite was dusted over the leaves before they were rubbed, which, as with other viruses tested (Kalmus & Kassanis, 1945), increases the dilution end-point and numbers of local lesions by factors of between 10 and 100 (Table 4) (Pl. 10, fig. 7).

The thermal inactivation point was found by heating 2 ml. samples of sap from infected tobacco plants for 10 min. at various temperatures, and then inoculating them to tobacco and bean plants. Table 5 shows that the thermal inactivation point of the yellow strains was 55–60° and of the others 65–70°, but the local-lesion counts show that much infectivity with all four strains was lost by heating for 10 min. at temperatures below 50°C. This fact suggests that cucumber mosaic virus is one of those that has a small temperature coefficient for heat inactivation and that loss of infectivity is not closely correlated with protein denaturation (Bawden, 1943). Other viruses with this property, for example, tomato bushy stunt and tobacco necrosis, can be inactivated by longer heating at temperatures well below that at which they are

inactivated in 10 min., and this is also true of cucumber mosaic virus. When 2 ml. samples of sap from tobacco plants infected with the spinach strain were heated for various lengths of time at 40° C., and then inoculated to tobacco, the average

TABLE 3. *Dilution end-point of four strains of cucumber mosaic virus*

	Strains							
Dilution	Spinach		Primula		Turnip		Yellow	
Undiluted	5*	121†	5*	133†	5*	97†	5*	87†
10^{-1}	5	100	5	115	5	114	5	67
10^{-2}	5	10	5	9	5	15	2	16
10^{-3}	4	2	3	1	4	3	1	2
10^{-4}	2	1	1	0	2	0	0	0
10^{-5}	0	0	0	0	0	0	0	0

* Number of tobacco plants systemically infected out of five inoculated.
† Average number of local lesions per leaf on French beans, var. Bountiful.

TABLE 4. *The effect of 'celite' in increasing infectivity at various dilutions*

(The figures represent mean number of local lesions per half leaf on French bean, var. Bountiful.)

	Strains			
	Spinach		Yellow	
Dilutions	Celite	No celite	Celite	No celite
Undiluted	171	12·9	72·9	5·0
1 in 10	198	8·5	95·0	2·6
1 in 100	35·7	1·6	5·2	0·3
1 in 1,000	5·5	0	0·8	0
1 in 10,000	0·6	0	0	0
1 in 100,000	0	0	0	0

TABLE 5. *Effect of heat on strains of cucumber mosaic virus*

	Strains							
Temperature (°C.)	Spinach		Primula		Turnip		Yellow	
Unheated	10*	388†	10*	236†	10*	351†	10*	235†
40	—	268	—	208	—	298	—	234
45	10	83	10	118	10	157	10	68
50	10	5	10	20	10	5	9	1
55	7	2	10	3	7	2	4	0
60	7	2	10	2	3	2	0	0
65	2	1	3	0	3	1	0	0
70	0	0	0	0	0	0	0	0

* Number of tobacco plants systemically infected out of ten inoculated.
† Mean number of local lesions per leaf on French bean, var. Bountiful.

numbers of local lesions produced per leaf were 80, 52, 38, 26, 6, 2 and 0 for samples heated respectively for 0, 30, 45, 60, 120 and 240 min. With other viruses that behave in this manner, such inactivated preparations are still serologically active, but this

property could not be tested with cucumber mosaic virus, because repeated attempts to produce antisera against the various strains by injecting rabbits with infective sap have all failed. With viruses that have a small temperature coefficient of heat inactivation, other factors than temperature influence determinations of thermal inactivation points. It may be that the yellow strain is less resistant to heat than are the others, but it is equally possible that the lower thermal inactivation point merely reflects a lower virus content in the samples that were heated.

Transmission by aphids

Several species of aphids are known to act as vectors of cucumber mosaic virus, and Watson & Roberts (1939) showed that the number of plants infected was increased if the aphids were first starved and then given only a short feeding period on an infected plant before being transferred to healthy plants. This phenomenon applies also to the spinach strain, with which results can be more accurately assessed than with other strains, because infective aphids usually produce countable local lesions at their feeding sites. Table 6 shows the result of an experiment with *Myzus persicae* to test the effects on transmission of varying the duration of preliminary fasting and of infection feeding. Ten similarly treated aphids were placed on each test plant and allowed to remain there overnight. It will be seen that preliminary fasting increased the number of infections only when the infection feeding time was short, and 1 hr. preliminary fasting was as effective as 4 hr. With unstarved aphids, the number of infections increased with increased times of infection feeding up to 4 hr.

Table 6. *The effect of varying preliminary fasting on the ability of* Myzus persicae *to transmit the spinach strain*

Preliminary fasting time (hr.)	Infections	Infection feeding time 2 min.	15 min.	4 hr.	Total
0	Local	1*	6	8	15
	Systemic	1	3	3	7/30
1	Local	22	19	7	48
	Systemic	7	6	2	15/30
4	Local	23	21	6	50
	Systemic	7	7	2	16/30
Total	Local	46	46	21	—
	Systemic	15/30	16/30	7/30	—

* For each treatment ten plants were used and each plant was colonized with ten aphids.

Although with starved aphids the length of infection feeding is important in determining the numbers of transmissions, the length of the test feeding matters little. Most of the aphids that can cause infection do so within the first minute, and varying the duration of test feeding between 1 and 30 min. did not significantly increase the numbers of infections. Individual aphids infective with the spinach strain sometimes cause more than one local lesion, probably because they insert their mouthparts into the leaf more than once when they first start to feed on the

test plants. It seems probable that they do this within the first minute, for when insects were transferred to a second set of healthy plants after only 1 min. on a first set, out of eight that caused infection in the first plants only two caused infection in the second. When the period on the first plant was extended to 5 min., only one out of fifteen aphids infected the second plant. In aphids that are feeding, therefore, it seems that infectivity is usually lost within 5 min. and often in 1 min., a shorter time than that found for other non-persistent viruses like henbane mosaic and potato virus *Y* (Watson & Roberts, 1940). If aphids are prevented from feeding, they remain infective for longer than when they feed. To quote one experiment with the spinach strain: *M. persicae* were starved for 4 hr. before an infection feed of 2 min., when they were enclosed in a Petri dish for periods of 0, 5, 15, 30, 60 and 120 min. before being transferred to test plants; the numbers of lesions produced by twenty-five aphids receiving the different periods in the Petri dish were, respectively, 12, 13, 6, 4, 2 and 0.

All four strains were most efficiently transmitted by fasted aphids that had fed for only a short time on the infected plant, but there were consistent differences in the number of infections obtained with the strains. Table 7 shows that the yellow strain was much less readily transmitted than the others, and that less than 1 in 20 of the aphids transmitted this strain, even when they were first fasted for 4 hr. and then given an infection feed of 2 min. The difference in transmissibility by aphids between this and the other strains seems greater than the differences in infectivity of sap. Although a lower virus content in plants infected with the yellow strain may partly explain the difference, additional factors may also be involved.

Table 7. *Transmission by* Myzus persicae *of different strains of cucumber mosaic virus*

No. of aphids per plant	Systemic infection out of ten plants with different strains			
	Spinach	Primula	Turnip	Yellow
20	10	10	10	5
10	10	9	8	1
5	9	8	7	0
1	6	5	5	0
Total	35	32	30	6

The results in Table 7 show how the proportion of infected plants increases as the number of aphids per test plant is increased. Some workers with viruses that give few infections unless many insects are used per plant, have suggested that infection occurs from the cumulative effects of several sub-minimal infective doses of virus injected by different insects, none of which alone would have caused infection. Counts of the local lesions produced on plants colonized with different numbers of *M. persicae* from tobacco plants infected with the spinach strain did not support this idea, but agreed with the conclusions of Watson (1936) and

Storey (1939) that infections are local and independent of one another. When batches of nine plants were colonized with 1, 3, 9 and 27 aphids per plant, the numbers of lesions obtained were 7, 22, 65 and 129. Up to nine aphids per plant, the increase in numbers of lesions was proportional to the numbers of insects used, and above nine, it was proportionally less, whereas it should have been more were there any cumulative effects. Thus, increasing the numbers of insects increases the probability of transmission merely by increasing the chance of including one infective individual in the total.

Several species of aphids can transmit cucumber mosaic virus, but the different species differ widely in their efficiency as vectors. In a comparative test with the spinach strain, using twenty-five individual aphids of three species, all of which received a 2 min. infection feed, *Myzus persicae* caused thirty-nine lesions, *M. ornatus* seventeen, and *Macrosiphum euphorbiae* only five. The same order of efficiency was found in transmitting the primula strain; of twenty tobacco plants colonized with equal numbers of the three species, sixteen were infected by *Myzus persicae*, twelve by *M. ornatus* and four by *Macrosiphum euphorbiae*.

I wish to thank the Government of India for the grant of a research scholarship which enabled me to carry out the above work.

REFERENCES

Allard, A. H. (1918). The mosaic disease of *Phytolacca decandra*. *Phytopathology*, **8**, 51.

Bawden, F. C. (1943). *Plant viruses and virus diseases*, 2nd ed. Waltham, Mass., U.S.A.: Chronica Botanica Co.

Bawden, F. C. & Roberts, F. M. (1947). The influence of light intensity on the susceptibility of plants to certain viruses. *Ann. appl. Biol.* **34**, 286.

Bawden, F. C. & Roberts, F. M. (1948). Photosynthesis and predisposition of plants to infection with certain viruses. *Ann. appl. Biol.* **35**, 418.

Bennett, C. W. (1944). Studies on dodder transmission of plant viruses. *Phytopathology*, **34**, 905.

Costa, A. S. (1944). Quantitative studies with carborundum and its use in local lesion tests. *Phytopathology*, **34**, 288.

Doolittle, S. P. & Walker, M. N. (1922). Notes on cucurbit mosaic. *Phytopathology*, **12**, 42.

Doolittle, S. P. & Walker, M. N. (1925). Further studies on the overwintering and dissemination of cucurbit mosaic. *J. agric. Res.* **31**, 1.

Harter, L. L. (1938). Mosaic of Lima beans (*Phaseolus lunatus macrocarpus*). *J. agric. Res.* **56**, 895.

Hoggan, I. A. (1933*a*). Some factors involved in aphid transmission of the cucumber mosaic virus to tobacco. *J. agric. Res.* **47**, 689.

Hoggan, I. A. (1933*b*). Some viruses affecting spinach and certain aspects of insect transmission. *Phytopathology*, **23**, 446.

Johnson, J. (1927). The classification of plant viruses. *Bull. Wis. agric. Exp. Sta.* no. 76, 16 pp.

Johnson, E. M. (1930). Virus diseases of tobacco in Kentucky. *Bull. Ky agric. Exp. Sta.* no. 306, p. 285.

KALMUS, H. &. KASSANIS, B. (1945). The use of abrasives in the transmission of plant viruses. *Ann. appl. Biol.* **32**, 230.

KASSANIS, B. & KLECZKOWSKI, A. (1948). The isolation and some properties of a virus-inhibiting protein from *Phytolacca esculenta*. *J. gen. Microbiol.* **2**, 143.

LIMASSET, P. & MONTGREMIER, H. A. DE (1946). Sur une maladie à virus provoquant des déformations foliaires remarquables chez le tabac et la tomate. *Ann. des Epiphy*, N. S., **12**, 181.

MOGENDORFF, N. (1930). 'Fern leaf' of tomato. *Phytopathology*, **20**, 25.

PRICE, W. C. (1934). Isolation and study of some yellow strains of cucumber mosaic virus. *Phytopathology*, **24**, 743.

PRICE, W. C. (1935). Classification of southern celery mosaic virus. *Phytopathology*, **25**, 947.

PRICE, W. C. (1940). Comparative host ranges of six plant viruses. *Amer. J. Bot.* **27**, 530.

SAMUEL, G. (1931). Some experiments on inoculating methods with plant viruses and on local lesions. *Ann. appl. Biol.* **18**, 494.

STOREY, H. H. (1939). Transmission of plant viruses by insects. *Bot. Rev.* **5**, 240.

WATSON, M. A. (1936). Factors affecting aphis transmission of the virus Hyoscyamus III. *Philos. Trans.* B, **226**, 457.

WATSON, M. A. (1938). Further studies on the relationship between Hyoscyamus virus III and the aphis *Myzus persicae* (Sulz.) with special reference to the effects of fasting. *Proc. roy. Soc.* B, **125**, 144.

WATSON, M. A. & ROBERTS, F. M. (1939). A comparative study of the transmission of Hyoscyamus virus 3, Potato virus *Y* and cucumber virus 1, by three species of aphides. *Proc. roy. Soc.* B, **127**, 543.

WATSON, M. A. & ROBERTS, F. M. (1940). Evidence against the hypothesis that certain plant viruses are transmitted mechanically by aphides. *Ann. appl. Biol.* **27**, 227.

WELLMAN, F. L. (1934). Infection of *Zea mays* and various other Graminae by celery virus I in Florida. *Phytopathology*, **24**, 1035.

WELLMAN, F. L. (1935). The host range of southern celery mosaic virus. *Phytopathology*, **25**, 377.

EXPLANATION OF PLATE 10

Fig. 1. Tobacco leaves showing local lesions caused by the spinach strain transmitted by *Myzus persicae*.

Fig. 2. Early systemic infection of tobacco by the spinach strain of cucumber mosaic virus.

Fig. 3. Local lesions of the ringspot type produced in *Datura stramonium* by the spinach strain.

Fig. 4. Local lesions produced in sugar beet by the spinach strain of cucumber mosaic virus.

Fig. 5. Systemic mottle and leaf deformity in sugar beet produced by the spinach strain.

Fig. 6. Severe symptoms of 'fern-leaf' in tomato caused by simultaneous infection with tobacco mosaic virus and the primula strain of cucumber mosaic virus.

Fig. 7. Leaf of French bean, var. Bountiful, showing local lesions produced by the spinach strain of cucumber mosaic virus during the winter; left-hand half leaf inoculated with the aid of 'Celite', right-hand leaf without.

(*Received* 26 *July* 1950)

BHARGAVA—*Some properties of four strains of cucumber mosaic virus*

RESOLUTION OF STRAWBERRY VIRUS COMPLEXES

IV. THE LATENT PERIOD OF VIRUS 3 IN THE VECTOR

By I. W. PRENTICE* and TAMSYN M. WOOLLCOMBE

East Malling Research Station, Kent

From the time of first feeding on plants infected with strawberry virus 3, 10–19 days elapsed before *Capitophorus fragariae* became infective, a longer 'latent period' than any previously recorded for an aphid-transmitted virus. The time taken for aphids to develop infectivity after leaving infected plants decreased with increasing duration of the infection feed. Aphids which had fed for 16 days on an infected plant caused infection in the first day of test feeding.

Introduction

The separation of a persistent virus (virus 3) from strawberry plants with severe crinkle has been previously reported, and it has been suggested that this virus probably has a long latent period in the vector (Prentice, 1949). The results of subsequent experiments support this conclusion.

The term 'latent period' has been used by previous workers to denote the time which elapses between an insect's feeding on an infected plant and its being able to infect another plant. A certain minimum time of feeding on the infected plant is required by the vector to pick up virus and a minimum test feeding time on the receptor plant is required to transmit it. These periods have been called respectively the 'acquisition threshold' and the 'inoculation threshold' (Sylvester, 1949) and must be taken into account in determining whether or not a latent period occurs. (The term 'aquisition threshold period' will be employed here, as 'threshold' does not usually imply duration.) 'Latent period' will be used to mean the interval (longer than the inoculation threshold period) during which a vector, subsequently shown to be infective, is unable to cause infection after the acquisition threshold period is passed.

A priori it would appear that if an insect feeds on an infected plant for a long period the latent period will be passed while the insect is on the infected plant. Such an insect would then be able to infect as soon as it left the infected plant. Bawden, however, states that a study of published results leads to the conclusion that the latent period 'seems to start from the time the vectors leave the infected plant rather than from the start of feeding on it' and that the duration of the latent period is not affected by the length of time for which insects feed on the infected plant (Bawden, 1943, p. 76). (Here, of course, 'latent period' is being used in the sense of the actual time lapse detected in an experiment and not in the sense defined above.) The results of the present experiments conflict with the hypothesis that the latent period starts when the vectors leave the infected plant.

* Now at Plant Pathology Laboratory, Milton Road, Harpenden, Herts.

Experimental

The methods employed were similar to those previously described (Prentice, 1948). Apterous strawberry aphids (*Capitophorus fragariae* Theob.) were allowed to feed on an infected plant (the *infector*) for a period (the *infection feeding period*); a number were then transferred to a healthy plant (the *indicator plant*) for the *first transfer feed* and moved to other indicator plants for the second and subsequent transfer feeds.

The number of aphids per indicator as shown in the tables is the number transferred to each plant for the first transfer feed. Most of the experiments extended over 2 weeks or more, and during this period some of the aphids developed wings. Alate aphids were not normally transferred to the next set of indicators; in addition, a number of aphids died or were lost during the experiment. Thus, in many experiments there were fewer aphids per indicator in the later transfers. In each experiment the infector was a *Fragaria vesca* plant infected with virus 3, and the indicators were symptomless *F. vesca* plants.

In earlier experiments (Prentice, 1949) virus 3 was transmitted after infection feeding periods of 6 days or more, but not after infection feeding periods of 1 or 4 days. With extended transfer feeding periods, transmission has now been obtained after infection feeding periods of 24 hr. (see Tables 1 and 2).

Table 1. *Experiment* 1

(Infection feeding period, 24 hr. Ten aphids per indicator.)

Transfer	Period (days)	Plants infected
1	10	0/5*
2	12	1/5

* Numerator shows number of plants infected, denominator shows number receiving the treatment.

Table 2. *Experiment* 2

(Infection feeding period, 24 hr. Five aphids per indicator.)

Transfer	Period (days)	Plants infected
1	9	0/5
2	8	1/5
3	2	1/5

In other experiments, aphids after an infection feeding period of 24 hr. were transferred to indicators for a total of 14 days, and aphids after an infection feeding period of 4 days were transferred for a total of 14 days, but no infections occurred.

Six alatae removed from the indicators of the first transfer feed in Exp. 1 were transferred to a *F. vesca* plant and enclosed for 12 days by means of a covered lamp glass. This indicator developed symptoms of infection. In another trial, five alatae from a stock of aphids which had fed for 14 days on a plant infected with virus 3 were transferred to a *F. vesca* plant for 2 days and then retransferred to a second *F. vesca* plant. Both plants developed symptoms of infection. It is concluded that alate aphids can transmit the virus.

Young aphids born on the indicators in Exps. 1–4 and totalling approximately 180 were transferred to a number of indicators. All remained healthy and there is thus no evidence from this investigation that the young produced by infective aphids are infective.

It was known from earlier experiments that virus 3 could be transmitted after infection feeding periods of 6 days or more. Experiments were therefore made to determine how soon aphids could transmit infection after leaving infectors on which they had fed for 6 days (see Tables 3 and 4).

TABLE 3. *Experiment* 3

(Infection feeding period, 6 days. Four aphids per indicator.)

Transfer	Interval (days)	No. of aphids on Plant 1	Plant 2	Plant 3	Plant 4	Plant 5	Plants infected
1	2	4	4	4	4	4	0/5
2	2	4	4	4	4	4	0/5
3	2	3	3	4	4	3	0/5
4	2	3	3	4	4	3	0/5
5	2	1	2	4	4	3	1/5 (no. 3)
6	2	1	2	4	4	3	1/5 (no. 3)
7	2	1	2	4	4	3	1/5 (no. 3)
8	2	1	2	4	4	3	1/5 (no. 3)
9	2		7		7		1/2 (no. 1–3)
10	2		6		6		1/2 (no. 1–3)
11	2		6		6		1/2 (no. 1–3)
12	2		2		1		1/2 (no. 1–3)

In Exp. 3, all the aphids remaining on the first three indicator plants of transfer 8 were transferred to one plant, and those on plants 4 and 5 were also transferred to one plant. Thus there were only two indicator plants in transfers 9–12. The results can be interpreted to mean that one aphid became infective during the fifth transfer feeding period and that this aphid survived until the end of the experiment, infecting each of the indicators on which it fed.

TABLE 4. *Experiments* 4–6

(Infection feeding period, 6 days. Five aphids per indicator.)

Transfer	Interval (days)	Plants infected Exp. 4	Exp. 5	Exp. 6
1	2	0/5	0/1*	0/5
2	2	0/5	0/5	0/5
3	2	0/5	0/5	0/5
4	2	0/5	2/5 (plants 2 and 5)	1/5 (plant 5)
5	2	0/5	1/5 (plant 2)	1/5 (plant 4)
6	2	4/5	—	—

* Twenty-five aphids on one plant.

Exps. 3–6 indicate that, after the completion of an infection feeding period of 6 days, a period of 6–10 days elapses before aphids become infective.

DISCUSSION

In Table 5 the results of previously published experiments with strawberry virus 3 are combined with those of the present experiments. Each successful infection that throws light on the length of the latent period is included separately. Series in which no infections occurred have been omitted.

TABLE 5. *Time elapsing before infections are produced after different infection feeds*

Infection feeding period (days)	Source of data	Interval (in days) elapsing before infections result	
		At least	Not more than
1	Table 2	9	17
	Table 1	10	22
6	Table 7*	4	8
	Table 4	6	8
	Table 4	6	8
	Table 4	6	8
	Table 3	8	10
	Table 4	8	10
	Table 4	10	12
	Table 4	10	12
	Table 4	10	12
	Table 4	10	12
8	Table 7*	2	4
	Table 6*	4	6
	Table 6*	4	6
	Table 7*	4	8
12	Table 5*	0	4
	Table 5*	0	4
	Table 5*	0	4
	Table 5*	0	4
	Table 7*	2	4
	Table 7*	4	8
	Table 7*	4	8
	Table 7*†	4	8
15	Table 6*	0	1
	Table 6*	1	2
	Table 6*	1	2
	Table 6*	2	4
	Table 6*†	4	6
16	Table 5*	0	1
	Table 5*	0	1
	Table 5*	0	1
	Table 5*	0	3
	Table 5*	0	3
	Table 5*	0	3

* Table number refers to Prentice, 1949.

† The form of the table referred to is such that the presence of this particular infection is obscured.

It will be seen that with an infection feeding period of 6 days, there is a delay of at least 4–10 days in different experiments before aphids become infective.

With an infection feeding period of 16 days the aphids are able to infect in the first day of test feeding. Thus the duration of the delay depends on the length of the previous infection feeding period. It seems therefore that with strawberry virus 3 the latent period can be passed while the aphids are feeding on the infector and that it should be considered as beginning when the acquisition threshold period is passed. Recent work by Maramarosch (Bawden, 1950, p. 104) indicates that this is true also for the latent period of the aster yellows virus in leaf-hoppers.

Exps. 1 and 2 show that the acquisition threshold period for this virus is 24 hr. or less. The time taken by individual aphids to pick up virus probably varies, but the error resulting from timing the latent period from the beginning of the infection feed instead of from the end of the acquisition threshold period is unlikely to be more than a day.

From the data of Table 5, the sum of the infection feeding period and the subsequent time before infections occur (i.e. the approximate true latent period) is not less than 10 and not more than 23 days. The *minimum* latent period lies between 10 and 19 days in different experiments, and the maximum between 12 and 23. Most of the results are consistent with a latent period of about 12–16 days from the commencement of feeding on the infector. It is, however, possible that the latent period is sometimes even longer and that some of the negative results in transmission tests are due to the termination of experiments before the completion of the latent period.

It is sometimes suggested that the latent period of a virus in an insect may represent the time required for the virus to enter the insect's blood and reach the saliva. The latent period of strawberry virus 3 is much greater than that previously recorded for viruses transmitted by aphids and is difficult to reconcile with such a theory. It has also been suggested (Watson, 1940) that with viruses which appear to have latent periods there may be no period after feeding on the infected plants 'during which the vectors are unable to transmit the virus, but merely a period of increasing infectivity towards a maximum'.

Insufficient trials have been made with strawberry virus 3 to say that no infections at all can occur during the presumed latent period. Consideration of the available data shows, however, that in experiments where the infection feed and test feed together totalled from 5–10 days, none of 150 test plants was infected; where the sum of the infection feed and the test feed was 11 or 12 days, one of fifty-five test plants was infected; where the total feeding period was from 13–16 days, twenty-one of 100 plants were infected; and where the total period was over 16 days, thirty-five of 105 plants were infected. The proportion of infections obviously falls off sharply as the combined infection feeding and test feeding time is reduced below 14 days. If any infections do occur with combined feeding periods of less than 10 days they are very few and it is probable that a true latent period exists in which no infections occur.

As the latent period can apparently be as short as 10 or 12 days, the occurrence of

infection, previously considered anomalous, in the first series of indicators in certain experiments (Prentice, 1949) no longer appears anomalous (although the high proportion of infections obtained in these series may be so).

REFERENCES

Bawden, F. C. (1943). *Plant viruses and virus diseases*, 2nd ed. 294 pp. Waltham, Mass., U.S.A.: Chronica Botanica Co.

Bawden, F. C. (1950). *Plant viruses and virus diseases*, 3rd ed. 335 pp. Waltham, Mass., U.S.A.: Chronica Botanica Co.

Prentice, I. W. (1948). Resolution of strawberry virus complexes. II. Virus 2 (mild yellow-edge virus). *Ann. appl. Biol.* **35**, 279.

Prentice, I. W. (1949). Resolution of strawberry virus complexes. III. The isolation and some properties of virus 3. *Ann. appl. Biol.* **36**, 18.

Sylvester, E. S. (1949). Transmission of sugar beet yellow-net virus by the green peach aphid. *Phytopathology*, **39**, 117.

Watson, M. A. (1940). Studies on the transmission of sugar-beet yellows virus by the aphis, *Myzus persicae* (Sulz.). *Proc. roy. Soc.* B, **128**, 535.

(*Received* 28 *September* 1950)

THE INFLUENCE OF DENSITY OF PLANT POPULATION ON THE INCIDENCE OF YELLOWS IN SUGAR-BEET CROPS

By J. W. BLENCOWE
Rothamsted Experimental Station, Harpenden, Herts

and T. W. TINSLEY*
Norfolk Agricultural Station, Norfolk

The incidence of yellows virus in sugar-beet crops was reduced by increasing the density of plant population. The variations in plant population were obtained by differences in row width and singling distance. The differences in susceptibility between large- and small-topped varieties, and between early and late sown crops, could not be attributed solely to differences in plant size. It is suggested that close planting would increase the yields of sugar beet and reduce the losses caused by yellows virus. Roguing infected plants during the early part of the growing season did not reduce the incidence of disease.

Although few experiments have been made to determine the effects of varying plant populations on the incidence of virus diseases, there are enough references in the literature to suggest that they may be considerable. Storey (1935) quoted observations in Africa which showed that close spacing of groundnuts reduced the incidence of rosette; interpretations attributing the reduction to effects on the insect vectors were considered by him to be premature. Clarke (1937) also observed in Tanganyika that the incidence of rosette was reduced when two rows of groundnuts instead of one were planted on each ridge.

The losses caused by two other diseases have been reduced by increasing the number of plants per acre; they are curly top in tomato (Shapovalov, Blood & Christiansen, 1941), and yellow-spot in pine-apple (caused by the tomato spotted wilt virus) (Linford, 1943). It is claimed that tomato spotted wilt was controlled in tobacco crops in South Africa by doubling or trebling the normal planting rate and then removing infected, and surplus healthy plants, after the invasion by thrips (van der Plank & Anderssen, 1944, 1945).

Van der Plank (1947) has pointed out that there is an implicit relationship between size of plant, planting rate and the spread of systemic pathogens. From single infection points, viruses invade whole plants, and, under constant conditions, increasing size increases the chances of the plants becoming infected. Usually the number of infective vectors entering a crop is limited, and increasing the plant population will reduce the proportion of the crop that will be infected from outside sources. This was demonstrated with tomato spotted wilt virus in crops in which it appears not to spread from plant to plant within the crop (van der Plank, 1946).

* Now at Rothamsted Experimental Station, Harpenden.

No tests have been made with diseases whose incidence depends not only on the number of plants infected from extraneous sources, but also on the spread within the crop.

The experiments described in this present paper were carried out at the Norfolk Agricultural Station, Sprowston, Norfolk. They were made to see how the amount and distribution of yellows and mosaic in sugar-beet crops were affected by various treatments influencing plant population and size of individual plants. Both yellows and mosaic viruses are transmitted by the aphids, *Myzus persicae* (Sulz.) and *Aphis fabae* Scop., of which the first appears to be the more important vector in the field, although the second is usually present in greater numbers on sugar-beet crops. The way in which the vectors have fed may influence the transmission of the two viruses differently; mosaic virus is optimally transmitted by aphids which, after having fasted, then feed for only short periods on infected plants; vectors soon lose their infectivity after leaving infected plants. Yellows virus persists in vectors for longer than mosaic virus and vectors are more likely to transmit, the longer they have fed on infected plants (Watson, 1946).

Experimental methods

The experiment was arranged to test all combinations of the following treatments:

(1) Wide rows versus narrow rows.

(2) Wide singling distance (i.e. plant spacing within the row), versus narrow singling distance.

(3) Large-topped variety versus small-topped variety.

(4) Early sowing versus late sowing.

(5) Addition of nitrogenous fertilizer versus no nitrogenous dressing.

(6) A roguing treatment in which plants showing symptoms early in the season, were removed from half the plots.

The experiment consisted of sixteen plots, each divided linearly into four subplots. Treatments which involved row-crop cultivations (row-width, variety and sowing date) were arranged on the sixteen whole plots. The other three factors (singling distance, nitrogenous dressing, and roguing) were allotted to the subplots in such a way as to confound the interaction $2 \times 5 \times 6$ with whole plots. Thus all combinations of the presence and absence of treatments (1) to (6) were represented on the sixty-four subplots. The experiment was divided into two blocks separated by a broad headland cropped with commercial sugar beet, the interaction $1 \times 2 \times 3 \times 4 \times 5 \times 6$ being confounded.

Experiment 1, 1946

The experiment was on a light soil which was deep-ploughed in November 1945. A basal manurial dressing applied in late March consisted of 3 cwt./acre agricultural salt, 3 cwt./acre superphosphate, and 1 cwt./acre muriate of potash. Sowing was preceded by seed-bed cultivations normal to the district. Subplots were

10 yd. by 10 yd. (approximately $\frac{1}{48}$th acre), giving a total experimental area of approximately $1\frac{1}{3}$ acres.

Treatments

(1) Row width: 30 in. versus 15 in. These widths were obtained by a standard Smythe seed-drill. Four coulters set at 15 in. were used, wide rows being obtained by closing alternate seed boxes. Three rounds of drilling completed each plot.

(2) Singling distance: 15 in. versus 10 in. Chopping out and singling were done by hand.

(3) Variety: Cannell's 937 versus Marsters. The first has strong-growing foliage, the second a small compact growth form.

(4) Sowing date: early April versus early May.

(5) Nitrogen: 4 cwt./acre sulphate of ammonia versus no nitrogenous dressing.

(6) Roguing treatments: plants showing symptoms before mid-July were removed from the appropriate plots and destroyed.

Experiment 2, 1947

The experiment was on a field similar to that of Exp. 1. Cultural operations were also similar, but subplot size was reduced to 1/100th acre.

Treatments

(1), (2) and (5) As in 1946.

(3) As the varieties used in 1946 were not available, Cannell's 22 (large top) and Kuhn P were used instead.

(4) Adverse spring conditions delayed sowing and only 3 weeks separated the two dates.

(6) Virus infection occurred later than in 1946 and roguing was delayed until late July.

Observations were made on the experiments at 3-weekly intervals during the summer. The numbers of plants infected with yellows or mosaic on each plot was recorded on each occasion, and a count made of aphid populations. This was done by selecting ten plants at random on each plot, examining them minutely to count and identify the aphids present.

The results are given below of the effects of the various treatments on the percentage of the plants infected with yellows, this being the customary manner of recording results in the field and the method that shows the practical value of any control measures. With a systemic disease, however, this does not necessarily show the full effects of the treatments on the spread of virus by aphids. It will do so when only a small proportion of the plants on all the plots are infected. When many plants on different plots are infected, the chances of further infections are reduced because many potentially infective punctures will be made by aphids feeding on plants that are already infected. The systemic symptoms of the disease provide evidence only of the first infection, any subsequent infection remains unrecorded.

The number of infective punctures necessary to infect any given percentage of a group of plants has been estimated mathematically and tabulated (Gregory, 1948). This Multiple Infection Transformation is used here to give a fuller picture of the extent to which virus was being spread on these experiments and to allow a more accurate comparison to be made between lightly and heavily infected plots.

Results

The main effects of the two experiments are summarized in Tables 1 and 2.

Table 1. *Sugar-beet spacing experiment*, 1946

Treatment	Plant no. (1000's per acre)	Infection (%)	No. of infective plant punctures (%)
A. Row width, 30 in. spacing; (control), 15 in. spacing	−10·8	7·91	10·2
V. Variety, Cannell's 937; (control), Marsters	− 3·2	7·61	9·6
S. Sowing date; early; (control), late	− 0·8	3·58	4·8
Standard error	± 0·60	±1·02	±1·35
N. Nitrogen, 0·8 cwt./acre	−1·0	3·63	4·4
R. Roguing, mid-July	0·7	−4·58	6·2
B. Singling distance, 15 in.; (control), 10 in.	− 2·2	4·57	−4·1
Standard error	± 0·90	±1·91	±2·89
Mean	21·0	14·19	18·2

Table 2. *Sugar-beet spacing experiment*, 1947

Treatment	Plant no. (1000's per acre)	Infection (%)	No. of infective plant punctures (%)
A. Row width, 30 in. spacing; (control), 15 in. spacing	−10·10	19·6	92·0
V. Variety, Cannell's 22; (control), Kuhn P	− 0·23	−6·3	−27·0
S. Sowing date, early 22 May; (control), late 30 April	− 0·53	11·8	49·5
Standard error	± 0·67	±2·04	±10·64
N. Nitrogen, 0·8 cwt./acre	− 0·55	−2·8	− 8·9
R. Roguing, late July	− 1·27	3·7	15·7
B. Singling distance, 15 in.; (control), 10 in.	− 1·83	7·7	36·3
Standard error	± 0·51	±2·85	±14·79
Mean	18·48	72·9	155·6

In 1946 the summer was cold and wet and there were few aphids on the plants. The highest mean population counted per plant was 0·3 *Myzus persicae* and 35 *Aphis fabae* in July; few alatae were observed. Yellows spread slowly during the growing season, and an average of 16% of the plants were infected at harvest.

There were more aphids in 1947. By mid-July there was an average of 60 *Myzus persicae* and more than 1000 *Aphis fabae* per plant. Large numbers of alatae were

produced which flew frequently during the warm dry weather. By the end of July an average of 60% of the plants showed symptoms of yellows, which then increased less rapidly; 80% of the plants were infected by early September. The growth of both roots and tops was adversely affected by the dry conditions during the summer; it is probable that all plants were infected with yellows by early October but much of the foliage had died and virus symptoms disappeared by this time.

Effects of treatments

Wide rows (1946). Wide rows decreased the plant population by 41%. The numbers of aphids recorded were very variable, the plant-spacing treatments produced no differences, either in mean numbers per plant or per plot, that were statistically significant. The percentage of plants infected with yellows was increased, especially when the plots were also wide-singled. The percentage of infected plants was also increased by a positive interaction between wide rows and large-topped beet (Cannell's 937).

Wide rows (1947). Although the percentage of plants infected with yellows was much higher than in 1946, the main effects were similar but none of the interactions was significant.

Wide-singling distance (1946, 1947). It had been expected that singling the plants at 15 in. instead of 10 in. would reduce the number of plants per acre by 33%, but apparently there were fewer accidental losses of plants on wide-singled plots, and plant population was reduced by only 10%. Percentage of plants with yellows was increased, especially when the rows were wide.

TABLE 3.

	1946				1947		
	Narrow singling	Wide singling	Mean		Narrow singling	Wide singling	Mean
	Percentages of plants infected with yellows						
Narrow rows	11·5	12·7	12·1	Narrow rows	58·5	69·7	63·1
Wide rows	17·4	22·6	20·0	Wide rows	79·7	85·8	82·7
Mean	14·5	17·7	16·1	Mean	69·1	76·8	72·9
	Estimated numbers of infective punctures per hundred plants						
Narrow rows	12·4	13·8	13·1	Narrow rows	95·1	124·2	109·6
Wide rows	19·6	27·0	23·3	Wide rows	179·9	223·4	201·6
Mean	16·0	20·4	18·2	Mean	137·5	173·8	155·6
	Plant populations per acre						
Narrow rows	29,350	25,294	27,322	Narrow rows	24,856	22,194	23,525
Wide rows	15,725	13,750	14,738	Wide rows	13,925	12,931	13,429
Mean	22,538	19,522	21,030	Mean	19,391	17,563	18,477

Table 3 shows the percentage of plants infected with yellows virus, together with the estimated numbers of infective punctures per hundred plants, on the four combinations of row width and singling distance in the experiments. The plant

populations are also given. The table illustrates the disparity between the observed percentages of plants infected and the estimated numbers of potentially infective punctures to attain these levels of infection.

Variety. In 1946 more of the large-top variety (Cannell's 937) became infected than the small-top Marster's variety, whereas in 1947 fewer of the variety Cannell's 22 (large-top) were infected than of Kuhn P, the small-top control.

Sowing date (1946, 1947). Late sowing increased the percentage of infected plants. *Aphis fabae* was more numerous on the late-sown plots.

Nitrogenous fertilizer. In 1946 the percentage of infected plants was increased on the plots treated with nitrogen, but in 1947 nitrogen had no effect.

Roguing. In 1946 the percentage of infected plants was reduced by roguing, especially on the wide-row plots. In 1947 roguing had no significant effect.

Sugar-beet mosaic. In 1946 too few plants were infected with mosaic for any comparative counts to be made. In 1947 the proportion of plants infected was again small, but the observations summarized in Table 4 show that the virus distribution on the plots of varied plant population was similar to that of yellows.

Table 4. *Percentages of plants infected with sugar-beet mosaic*

	Narrow singling	Wide singling	Mean
Narrow rows	0·9	1·5	1·2
Wide rows	5·4	5·9	5·7
Mean	3·2	3·7	3·4

Discussion

Van der Plank has proposed the hypothesis that, all other factors being constant, large plants are more liable to systemic infection than small ones (van der Plank, 1947). He suggests that differences in plant population may be regarded as differences in size, inasmuch as, where a systemic pathogen is carried by an active insect vector, the 'catchment zone' of the plant is its effective size. The observed effects of the plant population treatments in these experiments would agree with this concept, whereas the other treatments that affected the actual size of the plants had no consistent effect. In crops of large- and small-topped sugar beet it is possible that there are genetical differences which influence susceptibility to infection; although no great differences in resistance between varieties have been observed in the field. Similarly, late-sown plants are not only smaller than early-sown ones; they germinate and are singled later, they are physiologically younger, and may even support significantly different aphid populations.

Roguing treatments appear to be neither practical nor economical and it is uncertain if they reduce the incidence of disease. It has been observed that roguing may occasionally increase the numbers of plants infected with yellows, possibly because viruliferous aphids are disturbed when diseased plants are removed.

In seasons when all the plants in sugar-beet crops do not become infected with yellows, it is obvious that the reduction in the percentage of plants infected obtained

by closer planting might be of considerable importance. Even in conditions where all the plants do become infected, the time taken for this to occur would be greater on closely planted crops than on sparsely planted ones. Watson, Watson & Hull (1946), showed that the loss in yield caused by yellows virus increases with the length of time that the plant is infected, so any measure which postpones complete infection of the crop with the virus will be of value. Furthermore, the increased yield of sugar beet obtained by raising the plant population is important, even in the absence of virus diseases.

The authors wish to thank Mr F. Rayns and the members of his staff at the Norfolk Agricultural Station for their help and advice during these experiments.

REFERENCES

Clarke, W. C. (1937). The close planting of groundnuts. *E. Afr. agric. J.* **2**, 380.

Gregory, P. H. (1948). The multiple infection transformation. *Ann. appl. Biol.* **35**, 412.

Linford, M. B. (1943). Influence of plant population upon incidence of pine-apple yellow-spot. *Phytopathology*, **33**, 412.

Shapovalov, M., Blood, H. L. & Christiansen, R. M. (1941). Tomato plant populations in relation to curly-top control. (Abstract.) *Phytopathology*, **33**, 408.

Storey, H. H. (1935). Virus diseases of East African plants. III. Rosette disease of ground-nuts. *E. Afr. agric. J.* **1**, 206.

Van der Plank, J. E. (1946). A method for estimating the number of random groups of adjacent diseased plants in a homogenous field. *Trans. roy. Soc. S. Afr.* **31**, 269.

Van der Plank, J. E. (1947). The relationship between size of plant and the spread of systemic diseases. I. A discussion of ideal cases and a new approach to problems of control. *Ann. appl. Biol.* **34**, 376.

Van der Plank, J. E. & Anderssen, E. E. (1944). 'Kromnek' disease of tobacco; a promising method of control. *Fmg S. Afr.* **19**, 391.

Van der Plank, J. E. & Anderssen, E. E. (1945). 'Kromnek' disease of tobacco; a mathematical solution to a problem disease. *Sci. Bull. Dep. Agric. S. Afr.* no. 240.

Watson, M. A. (1946). The transmission of beet mosaic and beet yellows viruses by aphides; a comparative study of a non-persistent and a persistent virus having host plants and vectors in common. *Proc. roy. Soc.* B, **133**, 200.

Watson, M. A., Watson, D. J. & Hull, R. (1946). Factors affecting the loss in yield of sugar-beet caused by beet yellows virus. I. Rate and date of infection; date of sowing and harvesting. *J. agric. Sci.* **37**, 301.

(*Received* 4 *October* 1950)

SEROLOGICALLY RELATED STRAINS OF POTATO VIRUS *Y* THAT ARE NOT MUTUALLY ANTAGONISTIC IN PLANTS

By F. C. BAWDEN and B. KASSANIS
Rothamsted Experimental Station, Harpenden, Herts

Tobacco veinal necrosis virus is serologically related to potato viruses *Y* and *C*. It does not protect tobacco, *Nicotiana glutinosa*, or potato plants from infection by them, and tobacco and *N. glutinosa* plants infected with either virus *Y* or *C* are still susceptible to it. There is some evidence that it does not multiply normally in potato plants infected with virus *Y* and that it is sometimes suppressed in such plants.

The possession of antigenic groups in common with viruses *Y* and *C* is considered to justify identifying tobacco veinal necrosis virus as a strain of virus *Y*, and to be of greater taxonomic significance than failure to protect plants against other strains. A further difference from other strains is that it is more virulent towards tobacco than towards potato.

We have shown previously (Bawden & Kassanis, 1947) that potato virus *Y* occurs in a range of strains which are readily differentiated by the type and severity of symptoms they produce in different potato varieties. All the strains we then described were serologically related and mutually antagonistic; in other words, any strain was specifically precipitated by antiserum prepared against any other and plants systemically infected with any one resisted invasion by any other. The different strains caused widely different diseases in different potato varieties, but all were more virulent towards potato than towards tobacco. All produced necrosis in one or more of the potato varieties that were infected, whereas none produced necrosis in tobacco plants. There was some difference between the severity of the disease caused in tobacco plants by individual strains, but all produced symptoms of the same general type, a reduction of growth, with leaf symptoms showing initially as a 'vein-clearing' of the youngest leaves and later as an inter-veinal chlorosis with the appearance of dark green bands along the veins. The purpose of the present paper is to describe the behaviour of a further strain, which is anomalous in at least two respects. First, it is more virulent towards tobacco than towards potato; and, secondly, it is serologically related to the other strains but does not protect plants against them.

Symptomatology

The three strains of potato virus *Y* used in our experiments will be referred to as *Y* 1, potato virus *C* (*PVC*), and tobacco veinal necrosis virus (*TVNV*). The first two are derivatives of those already described under these names (Bawden &

Kassanis, 1947) and we have no evidence that their behaviour has changed since then. The third was kindly supplied by Dr W. R. S. Wortley, Commonwealth Potato Collection, Huntingdon Road, Cambridge, who isolated it from a naturally infected potato plant collected in South America. We consider it probable that this strain is similar to, and possibly identical with, a virus isolated by Nobrega & Silberschmidt (1944) from a plant of the Peruvian potato variety Serrana Negra, and we derive the name we use from their '*Necrose das Nervuras*'. The name records the most characteristic feature of the virus, namely the effect it causes in tobacco plants (*Nicotiana tabacum*, var. White Burley).

About 10 days after infection, the young leaves of tobacco seedlings become crinkled and deformed and their veins etched and necrotic. The initial reaction is severe, but the plants partially recover, and although they remain much stunted, the leaves produced subsequently show fewer necroses and some mottling. The whole symptom picture in tobacco closely resembles that produced by simultaneous infection with a strain of potato virus *X* and a strain of potato virus *Y* that alone causes 'vein-banding' (Smith, 1931), and *TVNV* may well be more widely distributed than the records of its occurrence suggest, but have been mistakenly diagnosed as such dual infections. The dual infections are readily distinguished, either by serological tests for potato virus *X* or by inoculation of sap to *Datura stramonium*, which is susceptible to potato virus *X* but not to *TVNV* or to any other strain of potato virus *Y* yet studied. Of the host plants we have tested, only tobacco reacts severely; in *Nicotiana glutinosa* and tomato (*Lycopersicum esculentum*, var. Kondine Red), as already described by Nobrega & Silberschmidt (1944), the effects are similar to those caused by other recognized strains of potato virus *Y*.

TVNV is readily transmitted to potato (*Solanum tuberosum*) plants by aphids or by mechanical inoculation of infective sap, but in the four varieties we have infected its effects are nil or slight. In the field most infected plants would escape detection unless transmissions were made from them to tobacco plants. Plants of the variety Majestic, whether infected in the current season or raised from infected tubers, behave like symptomless carriers. Likewise, Arran Pilot usually shows no abnormalities, although a transient faint mottle has been recorded in occasional plants. Infection in Craigs Defiance, in the first and subsequent years, is detectable by the production of a mild blotchy mottling, but there is no necrosis. Gladstone, which reacts with acute leaf-drop streak to all other strains of potato virus *Y* we have studied, also shows symptoms but is not seriously affected. Inoculated leaves become yellow and develop a few small necrotic spots, and systemically infected leaves show a definite but mild mosaic with occasional faint necrotic spots and streaks along the veins.

Our transmission tests, whether by aphids or mechanical inoculation of sap, to and from tobacco or other host species, have given no results suggesting that our culture of *TVNV* contained more than one virus or virus strain. Over a period of years it has consistently produced the same necrotic disease in tobacco seedlings.

In this respect our results agree with those of Nobrega & Silberschmidt (1944) and differ from those of Smith & Dennis (1940), who picture a disease that is apparently identical with tobacco veinal necrosis, which, however, they failed to reproduce consistently. Sometimes their infected tobacco plants developed only 'vein-banding' symptoms and further transmissions from such plants failed to reproduce the necrotic disease. They eliminated the possibility that the necrosis resulted from a dual infection with viruses *X* and *Y* and they tentatively identified the virus causing the 'vein-banding' symptoms as a strain of virus *Y*, but they reached no conclusion as to the cause of the necrotic disease. They did some plant protection tests, the results of which they describe as 'somewhat inconclusive', but which suggested that infection with potato virus *Y* did not protect plants against developing the necrotic disease. From the results they obtained by infecting potato plants, it seems probable that they were dealing with a mixed culture containing *TVNV* and another strain of potato virus *Y* similar to *Y* 1, and that occasionally *TVNV* was not transmitted when the other strain was.

Because of similarities in host range, properties *in vitro* and transmissibility by aphids, Nobrega & Silberschmidt (1944) suggested that *TVNV* might be a special strain of virus *Y*. They attempted to establish a relationship between the two by doing plant protection tests, but, like Smith & Dennis (1940), they described their results as inconclusive. With other strains of potato virus *Y*, including *PVC*, which is a striking anomaly in not being transmitted by species of aphids that are vectors of other strains, the phenomenon of mutual interference was readily demonstrable by plant-protection tests (Bawden & Sheffield, 1944; Bawden & Kassanis, 1947), and, if *TVNV* is a strain of virus *Y*, it seemed that its immunological behaviour is unusual. In an attempt to gain further information about its status, therefore, we have studied its serological relationship with *Y* 1 and *PVC*, and have also made detailed tests on the manner in which it interacts with them in a range of different host plants.

Serological tests

Antisera were made by injecting rabbits intraperitoneally with sap from tobacco plants infected separately with *Y* 1, *PVC* and *TVNV*. Leaves were picked from 3 to 4 weeks after infection when they were showing pronounced symptoms, macerated with a pestle and mortar, and the expressed sap was clarified by centrifuging for 10 min. at 8000 r.p.m. Injections were given weekly for 7 weeks, 5 ml. of clarified sap being injected each time, and the rabbits were bled a week after the last injection. Clarified sap from tobacco plants was also used as the test antigens in precipitin tests, which were made by mixing 1 ml. samples of antiserum and antigen at various dilutions in narrow tubes, which were placed in a water-bath held at 40° C. In these conditions clarified tobacco sap precipitates spontaneously after an hour or so, the time varying with different lots of sap, depending on the infecting virus or virus strain and on the age of the leaves, and it occurs more

rapidly in saline than in the presence of serum. Despite this complication, there are no difficulties in identifying a specific precipitate with virus *Y* antiserum; the specific precipitate separates sooner and is a different type, being flocculent and diffuse instead of granular and dense.

The injection of all three antigens produced antisera that precipitated specifically with clarified sap from plants infected with *Y* 1, *PVC* and *TVNV*, but not with sap from uninfected tobacco plants or with sap from plants infected with potato *X*, tobacco mosaic, cucumber mosaic or tobacco etch viruses. This showed that *Y* 1, *PVC* and *TVNV* are serologically related, a fact that on current usage justifies regarding them as strains of one virus. Such precipitin reactions do not demonstrate that the strains are serologically identical, but only that they have some antigenic groups in common. By analogy with other viruses, such as potato *X* and tobacco mosaic, it is likely that potato virus *Y* will be a complex antigen and that the individual strains will contain some specific antigenic groups (Bawden, 1950), but we have no positive evidence for such differences. That they may exist is suggested by the fact that, when the antisera were titrated against their homologous and heterologous antigens, they gave higher precipitin titres with the homologous antigens, but we were unable to confirm this suggestion by cross-absorption experiments. However, it is difficult to make critical absorption experiments, because of the problems involved in obtaining purified preparations of virus in sufficient quantities. Our tests were made with clarified infective sap, 9 ml. of which was thoroughly mixed with 1 ml. of undiluted serum. The mixtures were left for 8 hr. at room temperature when the specific precipitate was removed by centrifugation. After storage at 1° C. over-night the mixtures were heated for 10 min. at 60° C., to coagulate the normal plant proteins, when they were again centrifuged. Such absorptions with sap from uninfected tobacco plants removed no specific antibodies and left the precipitating power of the sera unaffected. When absorptions were made in this way with sap from plants infected with any of the three strains of virus *Y*, however, whether a heterologous or homologous serum was used, the absorbed serum failed to precipitate specifically when mixed with fresh lots of infective sap. The precipitin titres of the unabsorbed sera were from 1/80 to 1/320 and the absorbed sera were tested at a dilution of 1/10. This suggests that most of the antibodies in the sera were to antigens common to the three strains, and that, if there were any to antigenic groups specific to individual strains, these were quantitatively insignificant. In other words, it seems that these three strains, despite their wide differences in other respects, are antigenically very alike, and we have no reason to assume that *TVNV* differs antigenically more from *Y* 1 than does *PVC*. This is of interest because whereas *Y* 1 and *PVC* are mutually antagonistic in plants, *TVNV* and *Y* 1, or *TVNV* and *PVC*, are not.

Plant-protection tests

The failure of *Y* 1 and *PVC* to protect tobacco plants against *TVNV* is readily shown because of the characteristic necrosis caused by *TVNV*. We have made experiments at various times of the year in which batches of tobacco seedlings were divided into three lots, one of which was left uninoculated and the others infected, one with *Y* 1 and the other with *PVC*. Three to four weeks later, when the infected plants were showing typical systemic symptoms, all three lots were inoculated with *TVNV*, when invariably they developed typical symptoms of veinal necrosis. The presence of *Y* 1 or *PVC* did not appear to interfere in any way with the invasion of *TVNV*; necrotic lesions appeared in all three lots of plants simultaneously and the final symptoms in all were those of veinal necrosis.

The failure of the previous infection to influence the course or character of the disease caused by *TVNV* superficially resembles the result obtained when tobacco plants infected with *Y* 1 are infected with severe etch virus (Bawden & Kassanis, 1941, 1945), but there are significant differences. *Y* 1 not only does not interfere with the invasion by severe etch virus, but it is suppressed and supplanted from tissues in which it is already established. Also, plants infected with severe etch virus are immune from infection with *Y* 1. Neither of these features is true with *TVNV*. Although the symptoms of reinoculated plants were those of infection with *TVNV* alone, the plants still contained *Y* 1, which was shown by the production of leaf-drop streak in plants of the potato variety Majestic that were inoculated from them. So, too, when plants with symptoms of veinal necrosis were reinoculated with *Y* 1, there was no change in symptoms, but that *Y* 1 entered and multiplied was demonstrated by the fact that subsequent inoculations to Majestic produced leaf-drop streak. Serological tests suggested that the presence of *TVNV* did not appreciably influence the extent to which *Y* 1 multiplied. Sap from tobacco plants systemically infected with *Y* 1 usually gave a higher precipitin titre (1/8 to 1/32) than sap from plants with *TVNV* (1/4 to 1/8). When plants were infected with both strains simultaneously, or when plants with *TVNV* were reinoculated with *Y* 1, the resulting precipitin titres were usually higher than those from plants infected with *TVNV* alone and approximated or equalled those of plants with *Y* 1.

All three strains produce similar symptoms in *Nicotiana glutinosa* so that plant-protection tests are not readily made from observations on the symptoms of reinoculated plants. The production of a transient clearing of the veins in plants with *TVNV* when they were reinoculated with *Y* 1, however, suggested that the second virus became systemic. This fact was more clearly demonstrated by sub-inoculations to tobacco or Majestic potato plants. Inoculations to Majestic from plants first infected with *TVNV* and later reinoculated with *Y* 1 and *PVC*, produced necrotic local lesions, which with *Y* 1 were followed by the development of typical leaf-drop streak; similarly, inoculations from *N. glutinosa* first infected with *Y* 1 or *PVC* and then reinoculated with *TVNV* produced typical veinal necrosis in tobacco seedlings.

In the potato variety Majestic, which reacts to *Y* 1 and *PVC* with necrosis but which carries *TVNV*, the failure of *TVNV* to protect against the others is evident from the symptoms of reinoculated plants. Tests were made both with plants in the first year of infection with *TVNV* and with plants raised from infected tubers. Inoculations to these plants of both *Y* 1 and *PVC* produced local lesions in numbers comparable to those produced on previously healthy Majestic plants, and later *Y* 1 also produced systemic necrotic symptoms with some leaf-drop streak. In Arran Pilot, too, there was no protection, and plants systemically infected with *TVNV* developed more severe mosaic symptoms when reinoculated with *Y* 1, the presence of which was also identified by inoculation to Majestic. Two experiments with the variety Gladstone, one with plants infected 3 weeks previously with *TVNV* and one with plants raised from infected tubers, also gave results suggesting that *TVNV* did not interfere with the invasion of *Y* 1 or with the symptoms it caused. In a third experiment with this variety, however, plants that had been inoculated a month previously with *TVNV* failed to develop leaf-drop streak when inoculated with *Y* 1. The plants were rather old when they were reinoculated, but control plants of the same age became systemically infected with *Y* 1, though symptoms were less severe than is usual in younger plants. The progeny of the reinoculated plants showed the mild symptoms characteristic of infection with *TVNV*, and inoculations from them to Majestic gave no evidence that they contained *Y* 1. This result could be interpreted as evidence of protection, but we think it more probable that *Y* 1 failed to infect systemically because the plants infected with *TVNV* were senile at the time of reinoculation and many of the inoculated leaves fell soon after they were inoculated.

Although it seems clear that the presence of *TVNV* does not protect potato plants against invasion by *Y* 1 or *PVC*, there is some evidence that other strains of virus *Y* may interfere with the multiplication of *TVNV*. Smith & Dennis (1940) found that passage of their virus culture through potato plants usually changed the symptoms it produced in tobacco from necrosis to vein-banding. We have already stated that their culture probably contained *TVNV* and a strain similar to *Y* 1, and it seems that propagation in potato encouraged *Y* 1 at the expense of *TVNV*. There is no evidence from their experiments that the potato plants became infected with both, but we have had comparable results with the varieties Majestic and Arran Pilot, from experiments in which plants already systemically infected with *TVNV* were reinoculated with *Y* 1. The progeny from all such plants showed symptoms typical of infection with *Y* 1, and inoculations from them to tobacco seedlings recovered *TVNV* from less than one-quarter, the rest giving *Y* 1 alone. Also, from experiments with the varieties Majestic and Gladstone, we have evidence that *TVNV* does not readily invade and multiply in plants already infected with *Y* 1. Twelve plants with *Y* 1 were reinoculated with *TVNV*, but we were unable to recover it later from any of the young leaves. The significance of this, however, is uncertain, for the plants were severely diseased when reinoculated with *TVNV*, matured

early and the inoculated leaves soon fell. Potato virus *X* becomes systemic when inoculated to plants infected with *Y* 1, which suggests that some specific effect prevented the invasion of *TVNV*, but there is no evidence about its nature.

Discussion

Two methods have been increasingly used to identify clinically distinct viruses as related strains. These are the demonstration of serological relationship and the demonstration of mutual antagonism or interference, usually shown by the protection of a plant against invasion by a virus because of previous infection with another. With one recent exception, the two methods have given the same results when applied to the same pairs of viruses; that is to say, serologically related viruses have also protected plants against one another. The two phenomena have been studied in most detail with potato virus *X* by Matthews (1949), who found the antigenic constitution of strains to be correlated with the degree of mutual antagonism shown by plant-protection tests. Strains which in his serum-absoprtion experiments were serologically indistinguishable, protected plants against one another completely, whereas protection was incomplete with strains between which serological differences were detected.

The one exception is the failure of cucumber viruses 3 and 4 to protect against tobacco mosaic virus, which Fulton (1950) has ingeniously demonstrated by showing that the local multiplication of tobacco mosaic virus in cucumber leaves is unaffected by previous infection with cucumber virus 3 or 4. This failure to protect might have been expected by extrapolation from Matthews's results, for although the cucumber viruses are serologically related to tobacco mosaic virus, their degree of relationship is much less than that between strains of potato virus *X* which Matthews found to give incomplete protection. Only a small fraction of the total antigens in tobacco mosaic virus occurs in cucumber virus 3, and absorption of antiserum by the heterologous antigen does not appreciably affect its ability to react with the homologous antigen (Bawden & Pirie, 1937).

Our results with *Y* 1, *PVC* and *TVNV* represent a discrepancy between the two methods that seems much wider. The antigenic differences between *Y* 1 and *TVNV* do not seem significantly greater than those between *Y* 1 and *PVC*, yet the first two are not mutually antagonistic and the second two are. For the smooth development of methods of classifying virus strains, such discrepancies are perhaps unfortunate, but were only to be expected as the number of viruses studied by the two methods increased. As yet, the only recorded example with plant viruses of extensive interference between serologically unrelated viruses is the inability of potato virus *Y* to multiply in plants infected with severe etch virus (Bawden & Kassanis, 1945). With animal viruses, on the other hand, there are many such examples, and the ability of one virus to render a host refractory to the development of another does not seem to depend on serological similarities or any obvious biological relationship (Henle, 1950). Nor is there any obvious reason why it should. A serological relation-

ship shows a similarity of structural configuration, but the mechanisms underlying interferences are ill understood and may be of varied types. Mutual interference may reflect the fact that viruses combine with the same receptor substances or with some other essential substrates in host cells. If this is so, then combination will presumably be between specific configurations on the virus particles and the host substances, and if these configurations are also the antigenically active groups, then a correlation between ability to protect and serological constitution would be expected. There is, however, no *a priori* reason for this to be so, and the antigenically active groups may not be those biologically active in host cells.

Interference phenomena, however, could have other causes. Indeed, the simplest explanation of the protection afforded by the systemic infection of a plant with one virus strain against another, is that cells contain only a limited amount of the materials required for the synthesis of a given species of virus and, if this has already been used in the synthesis of one strain, a second finds the cupboard bare. Where interference is not mutual, as with *Y* 1 and severe etch, it may occur because one virus deleteriously affects some host-plant constituent which is essential for the multiplication of the other. The apparent inability of *TVNV* to multiply in potato plants seriously affected by *Y* 1 may be a further example of such a phenomenon.

The fact that discrepancies occur between relationships indicated by serological tests and tests of mutual antagonism does not invalidate positive results from either. Because the sharing of common antigens reflects structural similarities within the virus particles themselves, serological tests must have a greater taxonomic validity than tests of mutual antagonism, the basis of which is ill understood. Hence we would classify *TVNV* as a strain of potato virus *Y*. The fact that it does not protect plants against other strains of potato virus *Y* does not affect the identification of other viruses as related strains because they are mutually antagonistic, for no plant viruses have yet been found to protect plants against one another unless they resemble one another closely in many other properties. Until examples of mutual antagonism have been demonstrated between plant viruses that are obviously unrelated, i.e. differ morphologically or widely in their physico-chemical properties, the usefulness of positive plant-protection tests seems unquestionable. Negative tests, on the other hand, are less valuable, and it may be that future work, perhaps by the application of serology, will show that viruses now thought to be distinct types because they are not mutually antagonistic, are related strains.

REFERENCES

Bawden, F. C. (1950). *Plant viruses and virus diseases*. Waltham, Mass., U.S.A.: Chronica Botanica Co.

Bawden, F. C. & Kassanis, B. (1941). Some properties of tobacco etch viruses. *Ann. appl. Biol.* **28**, 107.

Bawden, F. C. & Kassanis, B. (1945). The suppression of one plant virus by another. *Ann. appl. Biol.* **32**, 52.

Bawden, F. C. & Kassanis, B. (1947). The behaviour of some naturally occurring strains of potato virus *Y*. *Ann. appl. Biol.* **34**, 503.

Bawden, F. C. & Pirie, N. W. (1937). The relationships between liquid crystalline preparations of cucumber viruses 3 and 4 and strains of tobacco mosaic virus. *Brit. J. exp. Path.* **18**, 275.

Bawden, F. C. & Sheffield, F. M. L. (1944). The relationships of some viruses causing necrotic diseases of the potato. *Ann. appl. Biol.* **31**, 33.

Fulton, R. W. (1950). Cross protection tests with cucumber viruses 3 and 4 and tobacco mosaic virus. *Phytopathology* **40**, 219.

Henle, W. (1950). Interference phenomena between animal viruses: a review. *J. Immunol.* **64**, 203.

Matthews, R. E. F. (1949). Studies on potato virus *X*. II. Criteria of relationships between strains. *Ann. appl. Biol.* **36**, 460.

Nobrega, N. R. & Silberschmidt, K. (1944). On a suspected variant of the potato virus *Y* (*Solanum* virus 2, Orton) which causes necrosis on tobacco plants. *Arq. Inst. Biol., S. Paulo*, **15**, 307.

Smith, K. M. (1931). On the composite nature of certain potato virus diseases of the mosaic group as revealed by the use of plant indicators and selective methods of transmission. *Proc. Roy. Soc.* B, **109**, 251.

Smith, K. M. & Dennis, R. W. G. (1940). Some notes on a suspected variant of *Solanum* virus 2. (Potato virus *Y*.) *Ann. appl. Biol.* **27**, 65.

(*Received* 9 *November* 1950)

EXPERIMENTS ON THE COLONIZATION OF POTATO PLANTS BY APTEROUS AND BY ALATE APHIDS IN RELATION TO THE SPREAD OF VIRUS DISEASES

By L. BROADBENT and T. W. TINSLEY
Rothamsted Experimental Station, Harpenden, Herts

(With 3 Text-figures)

Batches of potato plants in pots were placed in the field for limited periods among plants infected with potato virus *Y* and leaf roll virus. Some of the potted plants were surrounded by sticky boards which prevented apterous aphids from reaching them. Almost as many plants within the boards as without became infected, indicating that most of the spread of virus was by winged aphids.

Apterae were probably responsible for spreading the viruses throughout a hill after one or more stems were infected. They may carry infection to neighbouring plants, but most of these will have been infected already by alatae.

The number of plants contracting infection was unaffected by watering.

The spread of aphid-borne potato virus diseases from field to field must necessarily be by winged aphids, but Doncaster & Gregory (1948) have shown that in England such spread is negligible compared with that from infected plants within the crop. Although it was realized that alatae might also play a part, the local nature of the spread within the crop naturally incriminated the large populations of apterous aphids which develop on the plants in midsummer. However, when Doncaster & Gregory (1948) and Broadbent & Gregory (1948) found that much of the spread took place early in the season, before an apterous aphid population had developed, it became clear that apterae were probably not the principal vectors. Further circumstantial evidence that alatae were largely responsible was obtained when Broadbent (1950) showed that a high correlation existed between alate *Myzus persicae* (Sulzer) caught on sticky traps in the potato field and the amount of leaf roll infection; a similar but lower correlation existed for potato virus *Y*.

The experiments described in this paper were made to obtain information on the relative importance of apterae and alatae as vectors of viruses within the crop. They show that alatae are largely responsible for the local spread within the crop as well as for the spread from crop to crop over a distance.

1947 Experiment

Young healthy potato plants (variety King Edward) in pots were exposed in two rows of ten plants on each side of a double row of infected plants growing in the ground, alternately infected with leaf roll virus and potato virus *Y* (Fig. 1). Half the healthy plants were surrounded by sticky boards so that only alatae could

TABLE 1. *Number of* Myzus persicae *per plant at the end of the period of exposure, and virus infection*, 1947

	Mean no. of *M. persicae* per plant	No. of plants (1948 check)	Percentage		
			Healthy	Virus *Y*	Leaf roll virus
Batch 1 (4–19 June):					
Within boards	0	19	79	5	16
Without boards	0	18	72	0	28
Total	0	37	76	3	22
Batch 2 (19 June–3 July):					
Within boards	0·3	19	63	16	32
Without boards	0·5	16	37	19	56
Total	0·4	35	51	17	43
Batch 3 (3–17 July):					
Within boards	59	16	13	63	69
Without boards	54	16	0	100	44
Total	56	32	6	81	56
Batch 4 (17–31 July):					
Within boards	128	18	0	89	33
Without boards	182	19	0	100	42
Total	155	37	0	95	38
Batch 5 (31 July–14 Aug.):					
Within boards	17	18	11	83	56
Without boards	144	11	0	91	73
Total	80	29	7	86	62

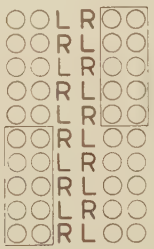


Fig. 1. Design of 1947 experiment. ○, healthy potato plant in pot; L, leaf roll, infected potato plants in ground; R, rugose mosaic (virus *Y*), infected potato plants in ground; □, sticky boards.

colonize them; the others were open to infestation by apterae from the diseased plants. The aphids infesting the plants were counted at the end of each period of exposure. The pots were sunk in the ground to prevent them drying out rapidly. Details for each batch are given below and in Table 1. About forty plants were put out on each occasion, but tubers were not formed on all, so records of health are not available for all plants.

Batch 1 (4–19 *June*)

No aphids were found on the thirty-nine plants at the end of their period of exposure, nor were any potato aphids caught on a nearby trap. Nevertheless, 22% of the plants were infected with leaf roll virus.

Batch 2 (19 *June*–3 *July*)

Few aphids were on the thirty-nine plants at the end of exposure: fifteen *Myzus persicae* (Sulzer), nine *Macrosiphum euphorbiae* (Thomas) and nine *Aphis rhamni* Fonsc. One *M. persicae* was trapped during this period. Again there was a considerable spread of disease, 43% of the plants contracting leaf roll and 17% virus *Y*.

Batch 3 (3–17 *July*)

Large migrations of *M. persicae* took place on 15 and 16 July, infesting nearby potato fields and the potted plants. When the plants were examined on 17 July every one was infested with *M. persicae*. Ten *M. persicae* were trapped during this period. Fifty-one *Aphis fabae* Scop., eight *M. euphorbiae* and seven *A. rhamni* were counted on the thirty-seven plants, in addition to 312 alatae, eighteen apterae and 1748 nymphs (mostly very young) of *M. persicae*. Nearby potatoes were lightly infested before this migration.

Batch 4 (17–31 *July*)

Large numbers of *M. persicae* were still flying when this batch was exposed, and all the plants were heavily infested with alatae when examined on 18 July. When taken in on 31 July the population was so heavy that counts were made on eight plants only, two in each row, one within and one without the sticky boards. On the eight plants nineteen *A. fabae*, eight *M. euphorbiae* and thirty-nine *A. rhamni* were found in addition to sixteen alatae, 183 apterae and 1041 nymphs of *M. persicae*. Predators and parasites attacked the aphids during this period; sixteen aphids were parasitized, seven syrphid larvae and eggs of coccinellids and chrysopids were found on the eight plants. Twelve *M. persicae* were trapped during this period.

Batch 5 (31 *July*–14 *August*)

When the plants were examined on 14 August it was apparent that all those within the boards were lightly infested, while those without were heavily infested with *M. persicae*. The comparative numbers in batch 4 suggested that some apterae had moved from the infected to the potted plants outside the boards, but batch 5 showed a greater movement by apterae. Counts on four plants from within and four without the boards gave:

		Alatae	Apterae	Nymphs
Myzus persicae	Within	1	4	63
	Without	1	82	492
Macrosiphum euphorbiae	Within	1	0	0
	Without	0	2	5
Aphis rhamni	Within	0	1	6
	Without	0	10	59

Eighteen *M. persicae* were trapped during this period, more than in the previous two periods when the potatoes became so heavily infested. This suggests that large numbers of alate *M. persicae* were flying but not reproducing on the potatoes. On the other hand, it might be that the population was kept in check by predators and parasites; forty parasitized aphids, two larval and two pupal coccinellids, two chrysopid larvae and many eggs of chrysopids and syrphids were counted on the eight plants.

The weather in 1947 favoured aphid migration, being warm, calm and dry over long periods (Table 2). Although sunk in the ground, the pots began to dry out in the middle of the fourth period and they were heavily watered on 27 July. The pots were again watered in the middle of the fifth period (8 August), but were very dry when lifted on 14 August.

TABLE 2. *Temperature and rainfall*, 1947

Batch	Mean temp. (°F.)	Rainfall	
		Inches	Days/exposure
1	56·3	0·867	7/15
2	61·3	1·522	5/14
3	60·3	1·006	7/14
4	66·3	0·509	3/14
5	63·4	0·068	4/14

Aphid counts on potatoes on a nearby farm are given in Table 3. *A. rhamni* was numerous on these, but few colonized the potted plants. The maximum population of *M. persicae* was reached during the first few days of August on the farm, and counts on the plants and on traps showed that large numbers of alatae left the field between 27 July and 8 August. There was no evidence that batch 5, exposed during this time, became heavily infested by alatae, as did batches exposed during the periods of maximum infestation on the farm in previous years (Broadbent, Chaudhuri & Kapica, 1950), but the greatest spread of leaf roll virus occurred during this exposure.

TABLE 3. *Aphid populations per plant on the potato field, Rothamsted Farm*, 1947

	Myzus persicae	*Macrosiphum euphorbiae*	*Aphis rhamni*
23 June	0	0	1
2 July	4	0·5	3
14 July	33	1·5	39
21 July	529	23	209
1 Aug.	1278	69	887
6 Aug.	911	55	465
14 Aug.	102	10	20
27 Aug.	31	0	32

Of the 170 plants exposed in batches 1–5, 29% remained healthy, 55% contracted virus *Y* and 43% leaf roll virus. In an experiment with Majestic potatoes on Rothamsted Farm the spread to the two plants nearest the infected plants (i.e. equivalent to the potted plants) was 26% virus *Y*, 88% leaf roll.

A considerable spread of leaf roll virus occurred in every batch in 1947 (Table 1); the only apparent differences between the conditions in this year and those in previous years, when few of the potted plants contracted leaf roll (Broadbent *et al.*, 1950) were adjacent infected plants and pots sunk in the ground, whereas previously they had stood on the surface. The pots sunk in the ground would not dry out so quickly as those standing on the surface, and it was thought possible that the water relations of the plants might have affected the transmission of the two viruses.

Effect of the barrier to apterae

Table 1 shows that the sticky barriers had little effect on the numbers of aphids on the plants until the late summer, when they stopped the apterae moving from the infected plants. Throughout the season the mean percentage virus records were:

	Within boards	Without boards
Remained healthy	34	24
Potato virus *Y*	50	60
Leaf roll virus	40	46

It may be deduced from these figures that alatae spread 83% of the season's spread of virus *Y* and 87% of the leaf roll. Data obtained by Broadbent & Gregory (1948) from different parts of England suggested that 58% of the season's spread of virus *Y* and 55% of the leaf roll was spread by the early alate migrants to the crops.

TABLE 4. *Percentage plants infected with potato virus Y and leaf roll virus in the inner and outer rows*, 1947

	Within boards			Without boards			Total		
	Remained healthy	Virus *Y*	Leaf roll virus	Remained healthy	Virus *Y*	Leaf roll virus	Remained healthy	Virus *Y*	Leaf roll virus
Inner rows	27	49	49	21	58	58	24	53	53
Outer rows	42	51	31	26	62	36	35	56	33

Table 4 gives the mean results for the inner and outer rows of potted plants. Whereas the same percentage infection with leaf roll virus and virus *Y* was obtained in the inner rows, in the outer rows the ratio of spread of virus *Y* to leaf roll was 1:0·59. The ratio for plants within boards was 1:0·61, without boards 1:0·58, so that the barriers made little difference to the decrease in spread of leaf roll to the outer rows; there was a difference of only 3% in the spread of virus *Y* to the inner and outer rows, compared with a difference of 20% for leaf roll. This difference for

leaf roll virus was large throughout the season, being 21, 20, 32, 19 and 13% in the five batches. If it is assumed that most of the infections came from the infected plants in the experiment this difference between outer and inner rows supports the hypothesis that leaf roll is spread (from plant to plant, not from field to field) under conditions that do not favour aphid flight (Broadbent, 1949). Smith (1931) states that to transmit leaf roll virus aphids must feed for at least 2 hr. on the healthy plant; they must have fed on a source of leaf roll virus for at least 6 hr. and then have spent about 48 hr. before they are capable of transmitting the virus. Thus aphids bred on the infected plants or visitors which had been there for more than 2 days would be suitably infective. At low temperatures or on windy days the mean flight length would be short and the first row of plants would be more likely to be reached than the second; the adverse conditions would favour the long feed necessary for transmission. With suitable flight conditions the aphids would probably move frequently from plant to plant, thus giving all the plants in the two rows equal chances of infection with virus *Y*. On the other hand, it is possible that the visitors, being older and therefore less active, were responsible for most of the leaf roll virus transmission, whereas those bred on the infected plants, being young and when weather conditions allowed, very active (Broadbent, 1949), would transmit most of the virus *Y*.

1948 Experiment

The previous experiments had suggested that leaf roll virus was not contracted by potted plants so often as might be expected (by comparison with the spread of potato virus *Y*) when pots stood on the soil surface and were usually dry. To test if the transmission of the two viruses was affected by the water relations of the plants, half the pots were sunk in the ground and watered every day; the other half were placed on the soil surface and not watered. So that there should be no great difference in height between the plants, those kept dry were placed in wide trenches. Forty plants each of two varieties, King Edward and Majestic, were exposed on each occasion, twenty of each being surrounded by sticky boards (Fig. 2). Every two rows of healthy potted plants were separated from the next two rows by two rows of infected potatoes planted in the ground, and infected plants surrounded the whole plot. Thus each healthy plant was adjacent to an infected one. It was known from previous experience that in these circumstances most of the potted plants would become infected, and the experiment was biased in favour of apterous aphids. The plants were spaced 27 in. between rows and 18 in. between plants. Three exposures were made, each for a period of 3 weeks. The water treatment of the plants was the same for all before and after exposure, so there was no difference in size when the plants were first put out.

Aulacorthum solani (Kalt.) and *A. rhamni* were scarce in 1948. Table 5 gives the records of *M. persicae* and *M. euphorbiae* and the percentage of plants which remained healthy and of those which contracted virus.

TABLE 5. *Aphids on the ten plants in each batch at the end of each exposure and percentage of plants which remained healthy and percentage which contracted virus*, 1948

(H=remained healthy; *Y*=potato virus *Y*; LR=leaf roll virus. Three exposures: (1) 21 days, 4–25 June, (2) 21 days, 25 June–16 July, (3) 21 days, 17 July–6 Aug. Al.=alatae, Ap.=apterae, Nym.=nymphs.)

	Within boards									Without boards								
	Myzus persicae			*Macrosiphum euphorbiae*			Virus			*Myzus persicae*			*Macrosiphum euphorbiae*			Virus		
Batch	Al.	Ap.	Nym.	Al.	Ap.	Nym.	H	*Y*	LR	Al.	Ap.	Nym.	Al.	Ap.	Nym.	H	*Y*	LR
Plants not artificially watered																		
King Edward																		
(1)	0	0	0	0	1	9	11	78	11	0	1	0	0	11	19	0	80	20
(2)	2	3	17	0	2	8	0	70	80	1	1	24	0	3	48	13	88	38
(3)	0	1	1	0	0	0	14	71	43	0	3	3	0	2	9	14	57	43
Total	2	4	18	0	3	17	8	73	46	1	5	27	0	16	76	8	76	32
Majestic																		
(1)	0	0	0	0	0	0	40	60	30	1	0	2	0	0	2	20	70	40
(2)	0	1	7	0	3	35	0	20	100	1	3	20	0	4	38	20	80	30
(3)	0	0	0	1	0	13	30	70	10	0	0	0	0	2	5	20	70	50
Total	0	1	7	1	3	48	23	50	47	2	3	22	0	6	45	20	73	40
Plants watered daily																		
King Edward																		
(1)	0	16	80	1	2	5	40	50	30	0	5	85	0	2	5	0	90	40
(2)	2	14	56	6	4	26	20	80	40	1	20	110	1	11	82	0	100	50
(3)	0	3	1	1	1	2	25	63	13	1	1	9	0	2	8	0	100	33
Total	2	33	137	8	7	33	30	61	26	2	26	204	1	15	95	0	97	41
Majestic																		
(1)	0	13	45	0	0	5	30	50	40	1	2	26	0	1	6	40	60	30
(2)	0	7	47	0	0	8	0	50	90	0	14	93	0	6	116	33	56	44
(3)	0	1	7	1	1	6	30	40	50	0	1	8	0	1	2	0	90	90
Total	0	21	99	1	1	19	20	47	60	1	17	127	0	8	124	24	69	55

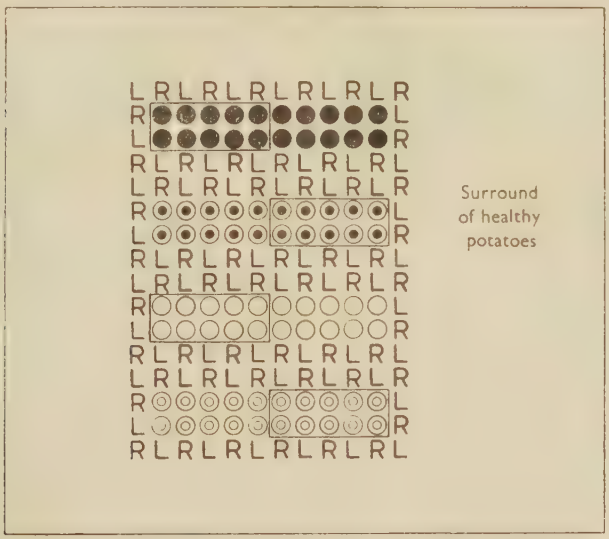


Fig. 2. Design of 1948 experiment. R, rugose mosaic (virus *Y*); L, leaf roll; all infected potato plants in ground. ○, pot on surface, unwatered; ●, pot sunk in ground, watered daily; healthy potatoes, var. King Edward. ◎, pot on surface, unwatered; ⊙, pot sunk in ground, watered daily; healthy potatoes, var. Majestic. □, sticky boards.

Batch 1 (4–25 *June*)

In this batch the 'dry' pots were protected by collars of tarred felt, so that little or no rain could get into the pots. The mean heights of the plants at the end of the period were:

	Dry (cm.)	Watered (cm.)
King Edward	16	26
Majestic	18	22

The dry plants made little growth and looked almost blue in colour by the end of the period. Although possibly unpalatable to *M. persicae*, they had contracted about the same amount of disease as the watered plants (Table 6). Though there was no evidence of apterous movement, more of the plants outside the boards became diseased (Table 7).

Table 6. *Total aphids counted at the end of the exposures, percentage plants remaining healthy and percentage diseased*, 1948

	Batch 1, 40 plants		Batch 2, 40 plants		Batch 3, 40 plants	
	Dry	Watered	Dry	Watered	Dry	Watered
Myzus persicae ...	4	273	80	364	8	32
Macrosiphum euphorbiae ...	42	27	141	260	32	25
Remained healthy (%)	18	27	8	12	21	14
Potato virus *Y* (%)	72	63	63	71	68	73
Leaf roll virus (%)	26	35	63	59	35	49

Table 7. *Total aphids counted on plants within and without the sticky boards at the end of the exposures, percentage plants remaining healthy and percentage diseased*, 1948

	Batch 1 (4–25 June), 40 plants		Batch 2 (25 June–16 July), 40 plants		Batch 3 (17 July–6 Aug.), 40 plants	
	Within	Without	Within	Without	Within	Without
Myzus persicae ...	154	123	156	288	14	26
Macrosiphum euphorbiae ...	23	46	92	309	26	31
Remained healthy (%)	31	15	3	16	26	8
Potato virus *Y* (%)	59	75	51	81	60	81
Leaf roll virus (%)	28	33	83	41	29	56

Batch 2 (25 *June–16 July*)

This batch of potatoes was bigger when exposed and the tarred felts were omitted. The King Edward plants averaged 35 cm. and the Majestic 31 cm. in height. Again there was no great difference between disease spread to dry and watered plants, though there were fewer aphids on the dry plants. Apparently there was a migration of apterae from the infected plants during this period and the spread of virus *Y* to plants within the boards was considerably less than to those without; however, more plants within the boards than without became infected with leaf roll virus.

Batch 3 (17 *July–6 August*)

The mean heights of the plants at the end of this period were:

	Dry (cm.)	Watered (cm.)
King Edward	19	34
Majestic	22	28

TABLE 8. *Total numbers of aphids and the percentage of diseased plants recorded during the three periods*, 1948

	Within boards	Without boards
Myzus persicae		
Dry	32	60
Watered	292	377
Total	324	437
Macrosiphum euphorbiae		
Dry	72	143
Watered	69	243
Total	141	386

	Within boards			Without boards		
	Remained healthy	Virus *Y*	Leaf roll virus	Remained healthy	Virus *Y*	Leaf roll virus
Dry (%)	16	61	46	15	75	36
Watered (%)	25	53	45	12	83	48
Total (%)	20	57	46	13	79	42

The effect of the sticky barriers

There was more apterous movement in 1948 than in 1947, especially by *M. euphorbiae*. Apterae walking to potted plants which were sunk in the ground would have fewer difficulties to overcome than those which had to climb down into the trenches and then up the sides of the pots before they could reach the dry plants. There was no difference between the numbers of *M. euphorbiae* infesting the dry and watered plants within the barriers, so the difference shown between the numbers infesting the plants without the barriers might be the result of the relatively greater isolation of the dry plants from colonization by apterae from the infected plants. The population of *M. euphorbiae* was not influenced by the water relations of the plants to the same extent as *M. persicae*. With *M. persicae* there was much less apterous movement, but a big difference between populations on dry and watered plants. However, this difference was not reflected in the number of plants which became diseased, perhaps because all the plants were treated similarly until exposed; the plants in the unwatered pots would not show the effects of drought for a few days.

More plants remained healthy within the barriers; the percentages with disease being:

	Within boards		Without boards	
	Virus *Y*	Leaf roll virus	Virus *Y*	Leaf roll virus
King Edward	67	37	87	37
Majestic	48	53	71	47

From these figures it may be deduced that, of the season's spread of virus *Y*, 77% in King Edward and 68% in Majestic was the work of alatae, while in both varieties the whole spread of leaf roll virus was due to alatae.

TABLE 9. *Total number of aphids counted on the three batches at the end of the exposures and the percentages of diseased plants in the varieties Majestic and King Edward*, 1948

	Majestic	King Edward
Myzus persicae:		
Dry	35	57
Watered	265	404
Total	300	461
Macrosiphum euphorbiae:		
Dry	103	112
Watered	153	159
Total	256	271

	Majestic			King Edward		
	Remained healthy	Virus *Y*	Leaf roll virus	Remained healthy	Virus *Y*	Leaf roll virus
Dry (%)	22	62	43	8	75	39
Watered (%)	22	58	58	13	81	35
Total (%)	22	60	50	11	78	37

TABLE 10. *Number of plants infested out of thirty-three examined on the basis of three leaves per plant; also trap catches; potato field, Rothamsted Farm*, 1948

	June				July				August	
	7	14	21	30	5	12	19	26	3	9
Myzus persicae	11	5	18	29	13	31	33	27	2	0
Macrosiphum euphorbiae	2	1	4	18	11	22	28	32	1	1
Aphis rhamni	7	10	14	13	10	14	20	10	9	0
Trap catches:										
Myzus persicae	2	4	3	6	3	4	15	1	0	1
Macrosiphum euphorbiae	0	3	1	1	0	4	6	3	3	1

The effect of the potato variety

There was no difference between the infestations of *M. euphorbiae* on the two varieties, but the King Edward plants had 54% more *M. persicae* than the Majestic (Table 9). The mean height of the King Edward plants was greater than that of the Majestic, and it is possible that a few more alatae were attracted to the taller plants (cf. Doncaster & Gregory, 1948). More of the Majestic plants remained healthy, fewer were infected with virus *Y* but more with leaf roll virus than the King Edward plants. The ratios were:

Majestic Virus *Y*:leaf roll = 1·00:0·83,
King Edward Virus *Y*:leaf roll = 1·00:0·47.

Table 10 shows the trap catches and aphid data on potatoes on Rothamsted farm, about 1 mile from the pot experiment. An estimation method (Broadbent, 1948) was used to assess the population. There was a heavy rainstorm on 4 July, and a count made on 5 July showed a big decrease in population. By 12 July, however, the population had recovered and the maximum was reached during the third week of July. If the plants exposed on 17 July became infested with aphids leaving the maincrop potatoes at this time, little trace of such infestation remained on 6 August, possibly owing to the predators and fungal and hymenopterous parasites which were noted on the farm crop at the end of July.

TABLE 11. *Temperature and rainfall*, 1948

Batch	Mean temp. (°F.)	Rainfall	
		Inches	Days/exposure
1	56·5	1·942	13/21
2	55·0	1·217	10/21
3	64·6	1·133	8/21

For comparison with previous years the relevant weather records during the periods of exposure are given in Table 11.

1949 EXPERIMENTS

To test further the relative importance of apterae and alatae in transmitting virus diseases, and also to find out if there was any difference in susceptibility between pot-grown and field potato plants, the following experiment was made.

A strip of infected plants, alternately infected with leaf roll virus and potato virus *Y*, was planted in the ground across the middle of the rows of a 13-row plot. There were five plants on either side of the infected plants in the rows, which consisted of two rows of potted plants (variety King Edward) alternating with a single row of normally grown plants of the same variety (Fig. 3). The plot was surrounded by guard plants, two rows on each side and nine plants at each end. Half the potted plants were planted at the same time as the plants in the ground (26 April); the other forty pots were planted a month later (31 May) and were placed in position on 15 June when about 1 in. high. Twenty pots in each batch were surrounded by sticky boards. The pots were sunk in the ground.

The plants within the boards were sprayed with nicotine at weekly intervals to ensure that no apterous population developed on them. In these circumstances it was assumed that all spread of disease to them was by alatae. The progeny of all the originally healthy plants in the plot was taken on 6 September, and five tubers from each plant were planted as usual the next year for disease records to be made.

The summer of 1949 was very hot and dry, and potatoes grew slowly. The plot was watered occasionally by an overhead rainer. An unusually large migration of *M. persicae* occurred during the early summer, but parasites and predators were

also abundant and only a small aphid infestation developed on potatoes. Counts of aphids on nearby plots of Majestic potatoes and on a sticky trap are given in Table 12. There was little difference between treatments in the spread of virus *Y* (Table 13) nor in the spread of leaf roll virus to potted plants within or without the sticky boards,

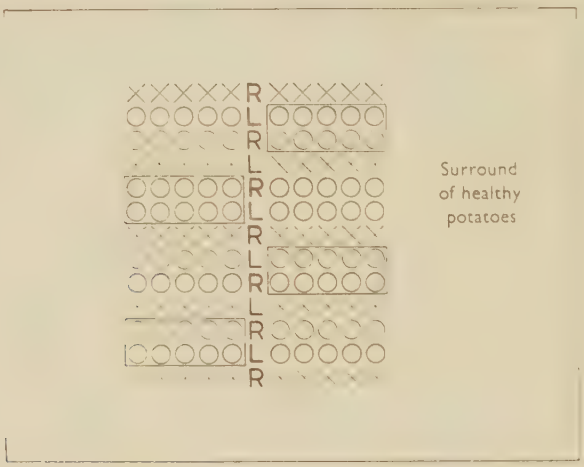


Fig. 3. Design of 1949 experiment. ×, healthy potato plants in ground; ○, healthy potato plants in pots; R, rugose mosaic (virus *Y*) infected potato plants in ground; L, leaf roll infected potato plants in ground; □, sticky boards.

TABLE 12. *Estimated number of aphids per potato plant on irrigated and non-irrigated plots*, 1949

	June		July				
	16	23	2	11	26	15 Aug.	7 Sept.
Irrigated plot:							
Myzus persicae	58	62	—	12	7	—	—
Macrosiphum euphorbiae	5	14	—	48	15	—	—
Aphis rhamni	9	9	—	85	150	—	—
Aphis fabae	1	2	—	93	23	—	—
Leaves per plant	41	56	—	100	150	—	—
Non-irrigated plot:							
Myzus persicae	84	61	10	14	4	0	0
Macrosiphum euphorbiae	6	8	13	27	18	3	2
Aphis rhamni	8	8	8	31	28	8	0
Aphis fabae	0	2	24	92	3	0	0
Leaves per plant	38	42	60	77	90	100	100

Aphids trapped per week near the pot experiment, 1949

	June				July				August					
	7	13	20	27	4	11	18	25	1	8	15	22	29	5 Sept.
Myzus persicae	3	3	5	6	4	2	2	2	0	0	0	0	0	0
Macrosiphum euphorbiae	0	0	1	3	1	0	1	0	0	0	0	0	0	0
Aphis fabae	8	17	35	217	972	753	668	526	137	1	0	3	0	1

either early or late planted. Of the plants in the ground, 10% contracted leaf roll virus, compared with 5% of those planted at the same time in pots. While this result supports those of the previous 5 years, when leaf roll virus was spread less to plants in pots than in neighbouring fields, spread of leaf roll virus in 1949 was so low that the results cannot be conclusive.

TABLE 13. *Hills infected in pot experiment*, 1949

	Plants in ground		Plants in pots: Within boards		Plants in pots: Without boards	
	Leaf roll virus	Virus *Y*	Leaf roll virus	Virus *Y*	Leaf roll virus	Virus *Y*
Planted 26 April	5/50	49/50	1/20	18/20	1/19	19/19
Planted 31 May	—	—	1/20	19/20	0/20	20/20
Total infection (%)	10	98	5	93	3	100

Five tubers from each hill were grown in 1950, and whereas all those from plants grown in the ground, and 193 of 195 from plants grown in pots without sticky boards, gave rise to plants showing virus *Y* infection, only 169 of 200 from plants within boards were infected.

A second experiment in 1949 was designed to test further the effect of the watering on the spread of virus *Y* and leaf roll virus. Twelve infected tubers were planted in each of two plots of Majestic potatoes; one plot was frequently watered with an overhead rainer, and the plants made rapid growth. The summer was very hot and dry, and the plants in the unwatered plot grew relatively poorly. Counts of aphids per plant on the two plots were similar (especially *M. persicae*), but hymenopterous parasites were so numerous that no large apterous population developed, despite the unusually large numbers of alatae which colonized the plants during June (Table 12).

Of the ten plants on either side of each of the infectors in the rows, 10% contracted leaf roll virus and 72% virus *Y* in the irrigated crop, 10% leaf roll virus and 66% virus *Y* in the non-irrigated crop. Thus there was no evidence of any differential spread of the two viruses in the two crops, but the effects of different levels of watering on aphid populations and the susceptibility of the plants to virus infection need further study.

DISCUSSION

It had been inferred from previous work that much of the spread of aphid-borne viruses in potato crops was caused by alatae; the experiments described above provide evidence that this is so. Apterous aphids move from plant to plant within the crop, especially when adjacent plants touch each other, as has been shown by a number of workers (e.g. Joyce, 1938; Czerwinski, 1943), but they probably carry infection only to neighbouring plants, most of which have been infected already by alatae.

Apterae may be responsible for spreading infection throughout a hill, once one or more of the stems has been infected. This is suggested by the 1949 experiment, in which only 85% of the tubers from plants within sticky boards were infected, compared with all the tubers from plants outside (those within boards were sprayed to prevent an apterous population developing).

Averaging the results of the 3 years' experiments with sticky barriers to apterous movement, it may be deduced that, of the season's spread of virus *Y* to neighbouring hills, about 83% was caused by alatae, which, on the other hand, caused 97% of the spread of leaf roll virus. This might account partly for the high correlation between alate *M. persicae* and the leaf roll infection, compared with the poorer correlation of numbers of alatae with virus *Y* infection (Broadbent, 1950). That apterae should play a larger part in spreading virus *Y* than leaf roll virus is surprising, as virus *Y* is non-persistent in the aphids, whereas apterae which have developed on a plant with leaf roll would usually remain infective for the rest of their lives.

REFERENCES

Broadbent, L. (1948). Methods of recording aphid populations for use in research on potato virus diseases. *Ann. appl. Biol.* **35**, 551.

Broadbent, L. (1949). Factors affecting the activity of alatae of the aphids *Myzus persicae* (Sulzer) and *Brevicoryne brassicae* (L). *Ann. appl. Biol.* **36**, 40.

Broadbent, L. (1950). The correlation of aphid numbers with the spread of leaf roll and rugose mosaic in potato crops. *Ann. appl. Biol.* **37**, 58.

Broadbent, L., Chaudhuri, R. P. & Kapica, L. (1950). The spread of virus diseases to single potato plants. *Ann. appl. Biol.* **37**, 355.

Broadbent, L. & Gregory, P. H. (1948). Experiments on the spread of rugose mosaic and leaf roll in potato crops in 1946. *Ann. appl. Biol.* **35**, 395.

Czerwinski, H. (1943). Untersuchungen und Beobachtungen über die Blattlaus *Myzodes persicae* Sulz. als Verbreiter des Kartoffelabbaues auf dem Versuchsfeld des Instituts für Acker- und Pflanzenbau Berlin-Dahlem und dem Versuchsgut Thyrow. *Angew. Bot.* **25**, 201.

Doncaster, J. P. & Gregory, P. H. (1948). The spread of virus diseases in the potato crop. *A.R.C. Report Series*, no. 7. London: H.M.S.O.

Joyce, R. J. V. (1938). On certain aspects of the feeding mechanism, feeding habits and ecology of the potato aphides, *Myzus persicae* Sulz. and *Macrosiphum gei* Koch, in relation to the dissemination of virus diseases of potatoes. M.Sc. Thesis, University of Wales.

Smith, K. M. (1931). Studies on potato virus diseases. IX. Some further experiments on the insect transmission of potato leaf roll. *Ann. appl. Biol.* **18**, 141.

(*Received* 4 *August* 1950)

FACTORS AFFECTING THE BEHAVIOUR AND ACTIVITY OF THE CABBAGE ROOT FLY (*ERIOISCHIA BRASSICAE* BCHÉ)

By MARY MILES
(*Wye College, University of London*)

(With 1 Text-figure)

The behaviour of cabbage root flies is governed by their need for food and shelter. Captive flies fed on sugar solution lived for periods up to 62 days, and a bred female laid 122 eggs.

Weather conditions determine the activity and the length of life of the flies. Feeding and egg-laying occur in warm sunny weather, and long periods of sunshine with temperatures of at least 60° F. in the latter half of April are associated with the onset of attack on the host plants. In cold or wet weather the flies shelter in soil or in thick herbage, and if they are immobilized for some time they die of starvation. In this way, the onset of cold weather checks the development of cabbage root fly attack. Details are given to show how the weather affected the activity of the flies in the springs of 1948–50 inclusive.

Eggs are laid in batches of varying size at irregular intervals during approximately 4 weeks. The position of the eggs about the host plants is affected by weather conditions. They are usually laid in the soil, but when the pressure of sunshine and drought is removed they are laid freely on aerial parts of the plants.

Knowledge of the influence of the weather on the activity of the flies enables attack to be anticipated with some degree of accuracy and control measures to be carefully timed.

Food requirements

Cabbage root flies in captivity soon die unless food is available. Schoene (1916) fed captive flies on banana and a syrup of sugar and water, and also placed blossoms in their cages. Smith (1927) fed them on sugar and water, and they mated and laid eggs in his cages. The writer also fed them on sugar and water. When kept singly in phials (3 in. × 1 in.) they had to be fed daily or they died. Under these conditions fertilized females lived for some time and laid eggs. During hot weather they were greatly excited when filter-paper moistened with sugar syrup was put in the phials and began feeding immediately. When kept without food, they became so weak that they were trapped by the film of moisture on the sides of the phials. When released and fed they again became active.

These observations suggest that nectar is an important factor in the natural diet of cabbage root flies. They have been seen at Wye in considerable numbers on the flowers of brassicas grown for seed and, according to Brittain (1927), they are fond of wild cherry bloom and they persistently return to it when driven away.

Influence of weather conditions on activity, feeding and length of life

The nature and quantity of food required by the flies makes them highly susceptible to the direct and indirect effects of the weather. Warm, sunny weather in the latter part of April encourages activity and enables them to feed in flowers and blossoms. Cold, wet, windy weather drives them to shelter in soil crevices (Brittain, 1927), thick herbage and crop waste. Since they die when deprived of food, enforced shelter during a long period of inclement weather results in the death of many through starvation.

The effect of dry, hot weather is more difficult to assess. Captive flies feed more often in hot weather, and it is reasonable to expect that wild flies have the same needs. But in such weather nectar-producing flowers are short-lived and other sources of moisture dry up, consequently less food is available. At these times the flies shelter in dense herbage, where the more humid atmosphere favours their survival.

Gibson & Treherne (1916) associated the length of life of the flies with the time of year. Under their conditions the length of life was 2–5 days for flies emerging in the period between 26 June and 1 July, and it increased to 7–25 days for those emerging in the period between 28 August and 27 September. At Wye summer temperatures had no ill effects on the length of life of captive flies when food and shade were provided. In 1949 the emergence of the summer generation of flies (mid-June to mid-July) was not associated with a peak of egg-laying in the field, and it was concluded that the prolonged hot, dry weather (rainfall between 15 June and 31 July was 0·44 in.) was the factor that limited activity.

The disappearance of the flies in the autumn seems to be the result of starvation induced by the onset of unfavourable weather. Flies emerged in captivity in the third week of September, and out of doors they were active in sunshine up to 12 October. Treherne (1915) recorded emergence as late as 27 September and found that activity persisted until 22 October. At Wye, egg-laying was observed in mild, sunny weather between 1 and 8 October in 1948 and 1949 but the onset of dull rainy weather terminated all activity. Flies provided with food survived low temperatures, but in the open the autumn generation was driven to shelter by the adverse weather and perished there.

Length of life and number of eggs laid

Little appears to be known of the numbers of eggs laid by cabbage root flies, but a chance mating among bred flies provided a record of egg-laying. The female emerged on 30 June and the male on 3 July. They were caged together from 4 July until their deaths on 10 August (♂) and 31 August (♀). Periodically the plant was removed from the cage and the numbers of eggs found were recorded (Table 1).

Table 1.

							No. of eggs
Between	6	July	and	13	July		31
,,	13	,,	,,	21	,,		51
,,	21	,,	,,	25	,,		0
				26	,,		1
				27	,,		6
,,	27	,,	,,	29	,,		9
,,	29	,,	,,	1	Aug.		0
,,	2	Aug.	,,	8	,,		12
,,	9	,,	,,	10	,,		1
,,	11	,,	,,	18	,,		4
,,	19	,,	,,	25	,,		7
						Total	122

The eggs were laid in batches of varying size at irregular intervals, the majority being deposited during the first 2 weeks after fertilization. From the point of view of cabbage root fly attack in the field, it is probable that only the first 2 weeks of egg-laying are important. If flies live to complete their egg-laying cycle, the later eggs contribute to the over-lapping of generations which has so frequently been recorded. This 2-week peak period of egg-laying corresponds approximately with the spring peak period of egg-laying in the field, which usually extends over 3 weeks. Any factor, such as adverse weather, that affects the life and activity of the flies during this period, reacts upon the intensity of attack in the field.

The association between weather conditions and egg-laying by the spring generation

Brassica plants were examined almost daily during April and May in 1948, 1949 and 1950 for the presence of eggs of cabbage root fly, and evidence was collected to show that the time and intensity of egg-laying were directly affected by weather conditions.

1948. Eggs were first found on 14 April, and the number of eggs and the proportion of plants infested increased until, on 21 April, eggs were numerous at all the plants examined.

Weather records show that on 10–13 April inclusive, the period immediately preceding the discovery of eggs, the sun shone for 8·8–11·5 hr. daily, and the temperatures rose until, on 13 April, the maximum day temperature reached 63° F. On 14 April there was little sunshine, but in the week following there were 5 days (15, 16, 18, 19 and 20 April) with more than 10 hr. of sunshine (a range of 10·1–11·3 hr.), and on 18–21 April inclusive the mean maximum day temperature was 70° F. (66–74° F.). Under these conditions the flies were very active.

1949. Eggs were first found on 20 April, 6 days later than in 1948, but this difference may be partly explained by a break (15–19 April) in the observations. On 25 April eggs were numerous about all plants examined.

Weather records again showed that during the period immediately preceding egg-laying (13–19 April) the weather was warm (max. day temp.* 65–80° F.) and sunny (7·7–13·3 hr. sunshine daily). Though maximum day temperatures were a little lower (61–67° F.) and there was some rain, the warm sunny weather continued for another few days (21–24 April inclusive) and the egg-laying flies maintained their activity.

1950. Eggs were first found on 22 April. As in 1948 and 1949, warm sunny weather preceded the finding of eggs. On the two days immediately preceding, 20 and 21 April, the maximum day temperatures were 61 and 64° F. respectively, and the sun shone for 9·3 and 10·0 hr.

On 23 April the weather was dull and cool. On 24, 25 and 26 April the maximum day temperature was only 46° F., and there were heavy showers of rain, sleet, snow and hail. The first eggs appeared to have been washed into the soil and no others could be found. On 27 April, there were 10 hr. of sunshine and the maximum day temperature was 53° F., but no flies were active about the host plants. On 28 April, the temperature reached 57° F. and there was intermittent sunshine, but no eggs were found. The following day (29 April) was wet. On 30 April, there were 10 hr. of sunshine and the temperature reached 62° F. On 1 May it was again warm (max. day temp. 67° F.) and sunny (10·7 hr. sunshine); flies were seen on the host plants and eggs were found in small numbers.

On 2–8 May, the weather was cooler (51–60° F.) with rain and very little sunshine (0–3·8 hr. daily). No flies were active and no increase in the number of eggs was observed. On 9 May the temperature rose again (max. day temp. 63° F.) and the sun shone for 10·5 hr., and females were numerous on the host plants. Warm, sunny weather (max. day temp. 63–67° F.; 8·8–14·2 hr. sunshine) continued for 5 days (9–13 May inclusive), and on 12 May eggs were seen at all plants examined.

Fig. 1 shows graphically that in spring there is a direct relationship between the onset of cabbage root fly attack in the field, as indicated by the intensity of egg-laying, and the prevailing weather. In the three consecutive seasons, 1948, 1949 and 1950, temperatures of over 60° F. and long periods of sunshine preceded the discovery of eggs in the field, and they became generally distributed in the crops under observation only when warm sunny weather lasted for several days. In 1948 and 1949 a prolonged period of high temperatures and bright sunshine occurred during the third and fourth weeks of April and was associated with intense egg-laying. In 1950 there was no prolonged period of warm sunny weather until the second week of May and eggs were not widely distributed until that time. These observations agree in principle with those of Brittain (1927) who found that in Nova Scotia there were rarely many eggs about the crops until after temperatures of 70° F. were reached. At Wye temperatures between 60 and 70° F. were sufficient to stimulate egg-laying when they were accompanied by long periods of sunshine.

* Maximum day temperatures for 1949 and 1950 are taken from records made at East Malling, 21 miles distant, because the thermometer at Wye was broken and could not be replaced.

The delay in the onset of attack in 1950 was not caused by the late emergence of flies, since they were seen to be active during the brief periods of warmth and sunshine that occurred earlier in the season, and they were found sheltering in adjoining crops of cereals and spinach. It was concluded, therefore, that the persistent cold and stormy weather immobilized them in shelter, and prevented feeding and egg-laying.

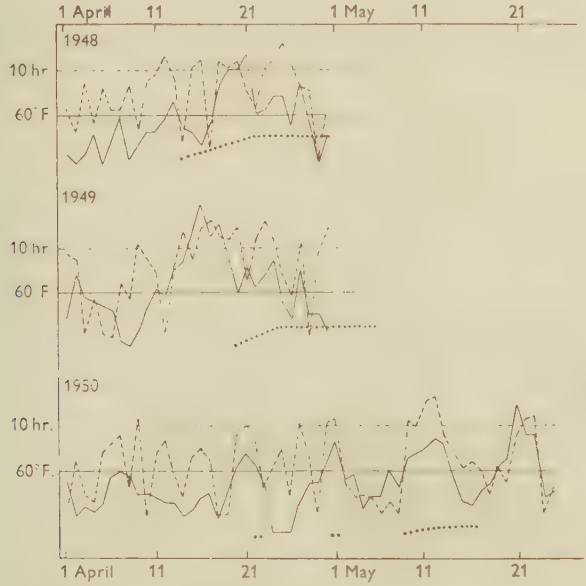


Fig. 1. ----- hours of sunshine; ——— maximum day temperature; ..... oviposition by *E. brassicae* Bché.

Comparison of larval populations in 1949 and 1950

The numbers of eggs about the host plants in 1950 were smaller than those noted in 1949 (Miles, 1950). Roots were examined on 3–5 June 1950 to compare the larval population with that observed in the same field at the same period in 1949. The plants did not come from the same part of the field, but the attraction of the plants and the mobility of the flies in favourable weather minimized the effect of small changes of site and the figures are roughly comparable. In 1949 the crop was cauliflowers and in 1950 it was cabbages, but both are equally attractive to the egg-laying flies (Miles, 1950).

The method of extracting the larvae resembled that used in 1949 (Miles, 1950), except that a sieve (60 meshes per in.) was used to remove fine particles of soil from the water in which roots were washed. This, together with the absence of peat from the soil around the cabbage roots, increased the efficiency of extraction.

In 1949, four plants, examined on 1–2 June, showed an average of sixty-eight larvae and puparia per plant. Another twelve plants, chosen at random from neighbouring plots unsuccessfully treated with insecticide and examined on 1–7 June, showed an average of forty-one larvae and puparia per plant. In 1950, twenty plants, examined on 3–5 June, had larval populations of 13, 55, 20, 7, 10, 7, 26, 21, 21, 6, 19, 2, 4, 7, 21, 7, 0, 5, 3 and 21 respectively, an average of 14 per plant.

These figures support the conclusion reached from field observations, that in 1950 egg-laying by the first generation of cabbage root flies was seriously affected by inclement weather.

Composition of immature populations

The composition of the immature population of *Erioischia brassicae* observed on 1–7 June 1949 differed greatly from that observed on 3–5 June 1950. In Table 2 the populations recorded above were analysed according to the stage of development. Larvae were described as mature if they had attained a length of 6–7 mm. The groups 'medium and small larvae' included first and second instars and third instar larvae that had attained a length of 5 mm. or less. Larvae of the three instars were readily distinguishable by means of a binocular dissecting microscope, but the rough classification adopted best expressed the differences in development, because at the time of examination in 1949 most of the larvae found were in the third instar. This instar varies greatly in length, Schoene (1916) recording a range of 2·5–8 mm.

TABLE 2. *Composition of immature populations of* Erioischia brassicae *in* 1949 *and* 1950

Date	No. of plants examined	Total no. of immature forms	Puparia		Mature larvae		Medium and small larvae	
			No.	Per-centage	No.	Per-centage	No.	Per-centage
1–2 June 1949	4	271	10	(3·7)	208	(77·1)	53	(19·2)
1–7 June 1949	12	489	15	(3·1)	355	(72·6)	119	(24·3)
3–5 June 1950	20	275	0	—	49	(17·8)	226	(82·2)

The data in Table 2 show that the development of *E. brassicae* in early June 1950 was far behind that for the same period in 1949, and provide further evidence of the check to egg-laying resulting from adverse weather in April 1950.

Influence of climatic factors on the position of the eggs

Investigators agree that in the field the eggs are usually laid in the soil about the stems of the host plants; they also occur in the leaf axils, on the petioles and on the undersides of leaves (Miles, 1950). The occurrence of larvae in the aerial parts of the plants, particularly the crowns of turnips and swedes, the inflorescence of cauliflowers and the hearts of brussels sprouts, has also been recorded in summer and autumn.

Flies in cages laid eggs indiscriminately on the surface of the soil and on the aerial parts of the plants, and the larvae fed freely in the mid-ribs of the leaves and in the axillary and terminal buds, as well as in the stem below soil level. Since the cages were not exposed to direct sunlight and the atmosphere within was humid, it was concluded that eggs were laid openly on the plants when the pressure of sunlight and drought were removed. In spring, when the weather is generally dry, there is little shelter about young plants and eggs are normally laid in the soil. Later in the season, when the larger plants offer more shelter to the flies and the atmosphere tends to be more humid, the eggs are laid in the open more frequently.

Discussion

The behaviour of cabbage root flies follows a pattern determined by the need for food and shelter. The activity of the flies, indicated by the intensity of egg-laying, is governed by factors that control feeding, the most important of which is the weather.

Other workers (Schoene, 1916; Glasgow, 1925; Brittain, 1927) have associated the occurrence of the flies and the beginning of egg-laying with the time of flowering of certain wild and cultivated fruits (*Prunus* spp. and related genera) in order to guide growers in their decisions about the time of application of control measures. The readiness with which captive flies feed on sugar solution suggests that wild flies may depend on nectar-producing flowers for their survival. An intimate association may therefore exist between the time of fly activity and the time of blossoming of shrubs and fruit trees. The observations recorded here show that the peak period of egg-laying begins about the end of the third week of April, a time when fruit trees and many wild and cultivated plants are in bloom. Since a short period of activity precedes egg-laying, it appears that the emergence of cabbage root flies may be expected about the middle of April.

With knowledge of the time of emergence of the flies and their reactions to weather conditions, the onset of attack in the field can be anticipated. Observations suggest that it begins in the first period of warm sunny weather after the middle of April. When this is delayed, as in 1950, the development of the spring attack is checked.

Since the onset of attack can be anticipated with some degree of accuracy, the application of suitable insecticides can be timed so that they give satisfactory results. Calomel dust (mercurous chloride) acts as an ovicide (Wright, 1940), and therefore it should be applied when weather conditions indicate that attack is imminent. If application is delayed, there is a risk that some eggs may hatch, for in warm soil the incubation period is reduced to 2 days. It has already been shown that the ovicidal properties of calomel are enduring (Miles, 1950), and further observations in 1950 have confirmed that an application at the beginning of the egg-laying period will give adequate protection to the crop, even when applied under unfavourable weather conditions.

A better understanding of the conditions under which attack takes place will permit greater flexibility in the use of control measures. The treatment of early cauliflowers can be deferred until the time of attack, and if, as in 1950, they are almost ready for cutting when infestation takes place, control measures may be unnecessary. The treatment of brassica seed beds can also be related to the stage of development of the seedlings. Since the time of attack can be anticipated it may prove to be more satisfactory to prevent injury in the seed bed than to cure it by dipping infested seedlings in insecticide. Visual symptoms of injury to summer-planted brassicas are usually the result of several unfavourable factors acting together. Infestation by cabbage root maggots is usually less intense in summer than in spring, but high temperatures and dry soil retard the establishment of the plants. The relative importance of these factors and the measures to be taken can only be decided locally.

This work was done while the writer was in receipt of a grant from the Agricultural Research Council. Prof. Herbert W. Miles has given advice and assistance throughout the investigation. Miss D. Johns prepared the graph for reproduction.

REFERENCES

BRITTAIN, W. H. (1927). The cabbage maggot. *Bull. Nova Scotia Dep. nat. Resources*, no 11.

GIBSON, A. & TREHERNE, R. C. (1916). The cabbage root maggot and its control in Canada. *Bull. int. Br. Can.* no. 12.

GLASGOW, H. (1925). Control of cabbage maggot in the seedbed. *Bull. N.Y. St. agric. Exp. Sta.* no. 512.

MILES, M. (1950). Observations on the biology and control of cabbage root fly. *Ann. appl. Biol.* **37**, 260.

SCHOENE, W. J. (1916). The cabbage maggot: its biology and control. *Bull. N.Y. St. agric. Exp. Sta.* no. 419.

SMITH, K. M. (1927). A study of *Hylemyia brassicae* Bouché. The cabbage root fly. *Ann. appl. Biol.* **12**, 312.

TREHERNE, R. C. (1915). The cabbage maggot: autumn development in British Columbia. *46th Ann. Rep. Ent. Soc. Ontario*, p. 130.

WRIGHT, D. W. (1940). Further experiments on the control of the cabbage root fly. *J. Minist. Agric.* **47**, 187.

(*Received* 10 *August* 1950)

WING COLORATION AS A MEANS OF DETERMINING THE AGE OF THE COLORADO BEETLE (*LEPTINOTARSA DECEMLINEATA* SAY)

By E. DUNN

*Ministry of Agriculture and Fisheries, Plant Pathology Laboratory, Harpenden**

(With Plate 11 in Colour)

The age of Colorado beetles can be determined, for at least 20–23 days after emergence, by means of the colour of the membranous hind-wings. The colour changes are described and a series of colour reproductions illustrate the appearance of a selection of wings during the first 25 days after emergence from the soil.

In countries where the Colorado beetle has not yet become an established pest it is very useful to be able to determine if a particular beetle is a first generation beetle which has emerged during the current season, or one from the previous year. A knowledge of the age of a beetle can often assist in deciding whether it has emerged locally or is an old beetle which has flown into the area.

In a preliminary report (Dunn, 1948) it was shown that it was possible to tell the age of a Colorado beetle for at least 14 days after emergence, by the colour of the membranous hind-wings. Further laboratory rearing experiments have shown that these colour changes follow a regular sequence of development which can be used to tell the age of the beetle for approximately 3 weeks after emergence.

Although the development of colour pigment in Colorado beetle wings has been studied in detail only in the laboratory, practical experience has proved that under natural conditions the same colour changes occur.

Shortly after emergence from the pupal instar first generation beetles usually leave the soil; a few, however, remain below ground until the following season. In parts of the Continent where conditions are favourable, a second generation of beetles may be produced late in the season. These do not usually emerge until the following spring. Such beetles have not been examined, and it is impossible to say if the same colour changes in the membranous wings apply under these conditions.

Description of colour change in wings

Pl. 11 shows a selection of wings taken from first-generation beetles bred in the laboratory.

It will be observed that for the first 8 days after emergence the wings are semi-transparent and practically without colour, except for the nervures which are yellowish. On the 10th day, however, the discal cell is beginning to show the

* Now at East of Scotland Agricultural College, George Square, Edinburgh.

presence of pigment and already a slight pink coloration can be detected in the small cell formed by the branches of the transverse nervure and the subcosta. By the 13th day the pink coloration of this small cell has become much deeper and the pigmentation in the discal cell slightly pink. The small cell formed by the branches of the transverse nervure and the sub-costa is always the first part of the wing to become red in colour and is very important in assessing the age of the beetle. By the 17th day this small cell has become distinctly red and stands out very clearly, while the pink coloration of the discal cell has become much deeper, especially along either side of the radial nervure. The centre of the cell, however, still remains practically colourless. From the 20th day onwards the red colour of the small cell and that of the discal cell becomes progressively deeper until even the centre of the discal cell has become quite red. There is little extension of the red pigmentation beyond the transverse and radial nervures before the 20th day, but by the 23rd day the pigmentation has extended into the apex and anal regions of the wing.

Included in Pl. 11 for contrast is a wing 25 days after emergence; a wing of a first-generation beetle emerged under natural conditions, about 20 days old; and a wing of a second-year beetle from the Continent. These demonstrate the variation in size of beetle wings under different environmental conditions and also the extent of the spread of the red coloration into the apex and anal lobes of the wing of a second-year beetle. Tower (1906) showed that temperature deviations could accelerate or retard the rate of pigment development within certain limits. Light is another factor of great importance in the development of colour in insects, but its effect on Colorado beetle wings is not known.

I am indebted to Mr V. Stansfield for the excellent colour photographs.

REFERENCES

DUNN, E. (1948). A method of distinguishing between young and old Colorado beetles. *Nature, Lond.*, **162**, 75.

TOWER, W. L. (1906). *An investigation of evolution in chrysomelid beetles of the genus* Leptinotarsa, pp. 158. Washington.

EXPLANATION OF PLATE 11

Colorado beetle wings showing appearance at different intervals of time after emergence from the soil.

Fig. 1. The appearance on (*a*) 1st day, (*b*) 3rd day, (*c*) 5th day.

Fig. 2. The appearance on (*a*) 8th day, (*b*) 10th day, (*c*) 12th day.

Fig. 3. The appearance on (*a*) 13th day, (*b*) 14th day, (*c*) 17th day.

Fig. 4. The appearance on (*a*) 18th day, (*b*) 20th day, (*c*) 23rd day.

Fig. 5. (*a*) The appearance on 25th day. (*b*) Wing of beetle emerged under natural conditions; about 20 days old. (*c*) Wing of second-year beetle from the Continent.

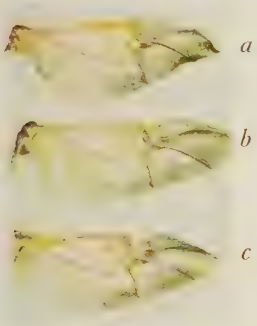


Fig. 1

Fig. 2

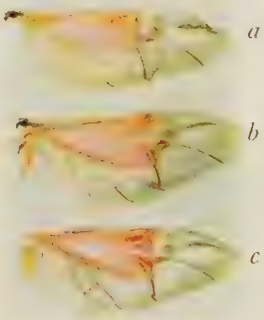


Fig. 3

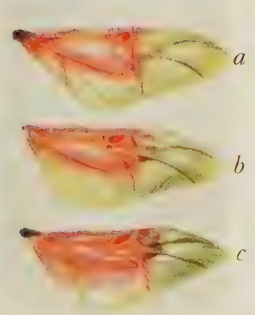


Fig. 4

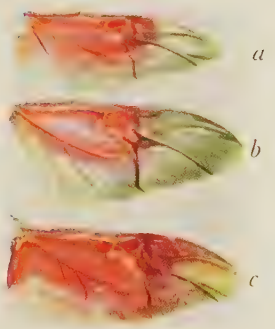


Fig. 5

THE EFFECT OF MANURES ON THE BOLTING OF THE BEET PLANT

By HAROLD H. MANN

Woburn Experimental Station, Husborne Crawley, Bletchley, Bedfordshire

In sugar beet, the effect on bolting of additions of potash manures, of superphosphate, and of the mixture of these was not significant, whether applied at the time of sowing or previously. Dung applied in the previous autumn slightly increased the number of plants which went to seed, and when applied just before sowing it had a more marked effect. An amount of sulphate of ammonia which would have approximately the same stimulating effect as the dung gave a slightly larger amount of bolting.

On red beetroot, it was possible to compare larger and smaller dressings of various coarse organic manures, and of each of these with and without dressings of sulphate of ammonia. In all cases the sulphate of ammonia and each of the organic manures largely increased the amount of bolting, and a doubling of the organic manure dressing or the addition of sulphate of ammonia to it caused a further increase. Sewage sludge gave an abnormally large amount of bolting, but it is doubtful whether this was due to the larger amount of organic matter and nitrogen applied in this manure. In general, any manurial addition causing more vigorous growth leads to an increase in bolting. There is a very large variation in the amount of running to seed in the crop of red beetroot from different parts of the same field, but the effect of the manures on the *proportion* which bolted was not widely different.

The general question as to the reason why beet plants should bolt more in one season than in another is discussed, and it is suggested that a check to the plants in an early stage, whether caused by dryness or waterlogging, or by low temperature, followed by a vigorous growing period *may* have something to do with the matter. This would agree with the experiences recorded in the present paper.

Ordinary stocks of all forms of *Beta vulgaris*, whether sugar beet, mangolds, or red beetroot grown as a vegetable, consist of mixed populations, mainly biennial, but containing a proportion of individuals which will 'bolt' under ordinary conditions of sowing in the open. In sugar beet, the selection of seed has been so careful that in England the number of plants which bolt during the growing season is very small in a normal year, amounting to not more than one or two per thousand in our experience at Woburn. With mangolds and red beetroot the breeding out of types with a tendency to a shortened life history has not been so complete, and, especially with red beetroot, which is normally lifted when quite young, there is, in a season favourable for growth, a tendency for quite a large proportion to go to seed if the plants are allowed to grow beyond a strict limit.

Hall (1928) considered that resistance to bolting was probably a complex genetic character, though one markedly affected by environment. Chroboczek (1934), in a study of the effect of various ecological factors on seed stalk development, found that, by raising the temperature at which the beet was grown, all bolting could be

eliminated, and came to the conclusion that 'when plants are grown under favourable conditions of temperature and light, they go to seed more readily when other environmental factors, such as nutrients and moisture supply, favour a vigorous development of the plant than when the conditions are unfavourable for vigorous growth'. He gives, however, no data on the effect of nutrients. The only data available on the effect of manures, are those furnished by the mangold experiments at Rothamsted, recently summarized by Watson & Russell (1943). They conclude that all fertilizer treatments tend to increase bolting, though there appeared to be no marked specific effects of the different nutrients. They say that 'any improvement in the nutritional status of the crop increases the tendency to bolt, so that there is a close relation between mean yield and bolting'. Of nitrogenous fertilizers nitrate of soda caused slightly more bolting than sulphate of ammonia except in the presence of dung, when the effect was reversed. Dung had a greater effect than any of the artificial fertilizers.

There have been a number of manurial experiments on sugar beet and on red beetroot at Woburn in recent years, from which it has been possible in some measure to judge the influence of manurial applications on the proportion of the plants which went to seed during a normal growing season, and some of these results may now be detailed.

Experiments with sugar beet

The number of bolters on many plots of sugar beet in various field manurial experiments have been counted for a series of years from 1929 to 1939. With this crop the number of plants which went to flower and seed was usually so small that no satisfactory conclusion on the influence of the manurial applications could be reached. The figures for such plants in the experimental fields from year to year are listed in Table 1.

Table 1. *Yearly numbers of plants in experimental plots*

Year	Variety	Date of sowing	No. of plants counted	No. of bolters per 1000 plants
1929	Kleinwanzleben I	23 May	38,200	1·13
1929	Kleinwanzleben II	23 May	29,400	0·75
1930	Johannsen P.	30 April	6,200	5·97
1932	Kuhn	6 and 12 May	38,600	0·36
1933	Kuhn	9 May	53,200	0·06
1934	Kleinwanzleben	30 April	45,500	2·70
1935	Kuhn	29 April	11,500	0·17
1936	Kleinwanzleben I	8 April	12,800	1·48
1936	Kleinwanzleben II	27 April	12,800	0·31
1936	Kleinwanzleben III	15 May	13,200	0·08
1936	Kuhn	23 April	11,600	1·29
1937	Kleinwanzleben E.	30 April	30,900	0·68
1937	Kuhn	29 April	12,900	2·02
1938	Kleinwanzleben E. I	13 April	9,600	29·79
1938	Kleinwanzleben E. II	13 April	41,500	33·16
1939	Kleinwanzleben	10 May	33,200	1·11

Except in one year, namely 1938, the amount of bolting is clearly insufficient to enable a fair judgement to be made of the influence of any treatment on the results. So far as the varieties used are concerned, we have:

(1) Johannsen P (only one case)—5·97 bolters per 1000 plants.

(2) Kuhn—variation between 0·06 and 2·02 bolters per 1000 plants, with a mean of 0·47 per 1000 plants.

(3) Kleinwanzleben—variation between 0·08 and 2·70 bolters per 1000 plants (if we exclude 1938) or a mean of 1·25 per 1000.

In 1938, using the same variety as in the majority of the above cases (Kleinwanzleben), a very different result occurred. The seed was sown about the normal time (13 April), but instead of getting only just over 0·1% of bolters, there were 3·25%, which is quite a large proportion of the total. It is so evident that this large number was a function of the season that it is worth while to give in more detail the characteristics of the season in question. The seed was sown in the middle of a long drought which continued from 27 March to 2 May, though there was enough moisture on 13 April to give good germination. Even after 2 May the conditions remained very dry till 27 May, after which there was a fairly dry but not very abnormal season. The temperature during the growing season was about average, so that the only special feature was the very considerable dryness of the soil during at least the early growth of the sugar beet. Under these conditions, the following proportion of bolters per 1000 plants was obtained with various manurial dressings.

Experiment I

	Bolters per 1000 plants
(1) *Effect of farmyard manure*, 10 tons/acre, ploughed into the land in the previous December	
No farmyard manure	30·7 ± 3·1
Farmyard manure, 10 tons/acre	35·7 ± 3·7
(2) *Effect of agricultural salt*, 5 cwt./acre, applied either in December, in January, in March or at sowing	
No salt	31·0 ± 4·9
5 cwt. salt/acre	35·4 ± 4·8
(3) *Effect of potash manures*, 1 cwt./acre muriate of potash, applied either in December, in January, in March or at sowing	
No potash manure	33·8 ± 3·3
1 cwt. muriate of potash per acre	32·6 ± 3·4
(4) *Effect of superphosphate*, at the rate of 0·5 cwt. P_2O_5/acre applied either in December, in January, in March or at sowing	
No phosphates	33·9 ± 4·2
0·5 cwt. P_2O_5/acre as superphosphate	32·7 ± 2·1
(5) *Effect of time of application* of salt, potash manure and superphosphate	
Fertilizers applied in December	32·5 ± 1·7
Fertilizers applied in January	33·4 ± 7·8
Fertilizers applied in March	30·9 ± 2·6
Fertilizers applied at sowing	35·5 ± 3·2

Experiment II

	Bolters per 1000 plants
(1) *Effect of farmyard manure*, at two rates (containing 0·8 and 1·6 cwt. nitrogen/acre) applied on 1 April	
No farmyard manure	18·4 ± 5·3
0·8 cwt. nitrogen in farmyard manure	15·8 ± 12·8
1·6 cwt. nitrogen in farmyard manure	30·3 ± 2·1
(2) *Effect of sulphate of ammonia*, at two rates (containing 0·4 and 0·8 cwt. nitrogen/acre) applied on 13 April at the time of sowing	
No sulphate of ammonia	19·1 ± 9·8
0·4 cwt. nitrogen in sulphate of ammonia	20·8 ± 14·7
0·8 cwt. nitrogen in sulphate of ammonia	34·6 ± 8·0
(3) *Effect of mineral manures*, at two rates. The mineral manures consisted of superphosphate and muriate of potash. The smaller rate contained 0·4 cwt. P_2O_5 and 0·5 cwt. K_2O/acre, and the larger double this amount. They were applied on 12 April	
No mineral manures	26·1 ± 14·5
0·4 cwt. P_2O_5 and 0·5 cwt. K_2O/acre	29·2 ± 13·7
0·8 cwt. P_2O_5 and 1·0 cwt. K_2O/acre	28·5 ± 11·1

Under the conditions of these two experiments, the effect of additions of potash, of phosphates, and of the mixture of these two classes of manures was not significant, whether applied at the time of sowing or previously. Dung applied in the previous autumn slightly increased the bolting, and when applied just before sowing it had a more marked effect. An amount of sulphate of ammonia which would have approximately the same stimulating effect as the dung gave a slightly larger amount of bolting, when applied at the time of sowing.

It is unfortunate that we have no other year in which conditions were as favourable to bolting as was 1938, but these figures substantially conform to what has been generally believed, namely, that, when the climatic conditions favour a tendency to bolt, the addition of manures which stimulate growth increases this tendency.

Experiments with red beetroot

While sugar beet is now highly selected, and bred so that it shall not bolt, other forms of *Beta vulgaris* have not lost the tendency to anything like the same extent, and the occurrence of a long-term experiment with globe beetroot, grown as a vegetable and hence lifted while still young, enabled a check to be made of at least certain forms of organic manure and of the addition of sulphate of ammonia with and without each of these. Records are available for 5 years, namely 1946, 1947, 1948, 1949 and 1950. In 1947 no bolting occurred in the beet when, owing to an unfavourable spring season, the crop was sown very late, and late planting also resulted in little or no bolting being seen in 1949. But the amount of seed stalk development in the other three years was considerable and in certain plots in 1950 was very large indeed. The seed was sown on 3 and 8 April, 6 April, and 31 March

respectively in the 3 years, so that there is little difference in the time of starting growth.

The experiment from which the records are taken was one designed to determine the relative utility of four coarse organic manures, with and without the addition of sulphate of ammonia, in converting a piece of poor sandy loam into regular market-garden land. Every year, therefore, the land was dressed, in February or thereabouts, with either 15 or 30 tons/acre of one of the following: (1) farmyard manure made by cattle and pigs in the yard attached to the Station; (2) and (3) compost made from farm refuse and straw, activated with either half its weight of dung or of sewage sludge; and (4) sewage sludge itself. Half of the plots with each manure had, in addition, 4 cwt./acre of sulphate of ammonia. Plots were also retained without any dressings at all, and with 4, 8 and 12 cwt. of sulphate of ammonia per acre without any organic manure. All plots received a basal dressing of phosphates and potash. It is thus possible to compare the amount of bolting which took place (1) when equal quantities of several coarse organic manures were added just before the beet was sown; (2) when the amount of each of the organic manures was doubled; (3) when 4 cwt./acre of sulphate of ammonia was added to the dressings of each of the organic manures; and (4) when the amount of sulphate of ammonia was doubled, and trebled, without any other addition.

The coarse organic manures were put on in equal quantity, irrespective of their composition, and so the actual amount of manurial constituents added per acre was different. The composition of each of them as applied, in 1948 and 1950 is shown in Table 2. Figures for the composition in 1946 are not available.

TABLE 2. *Composition of organic manures*

	Dry matter (%)	Organic matter (%)	Nitrogen (%)	Ash* (%)	Nitrogen in organic matter (%)
Farmyard manure:					
1948	23·2	14·2	0·50	9·0	3·52
1950	23·4	11·4	0·40	12·0	3·51
Compost made with dung:					
1948	33·8	12·8	0·58	21·0	4·53
1950	24·9	11·2	0·49	13·7	4·37
Compost made with sludge:					
1948	57·0	18·2	0·86	38·8	4·73
1950	40·3	16·8	0·76	23·5	4·52
Sewage sludge:					
1948	64·2	27·3	2·10	36·9	7·69
1950	64·8	27·3	1·48	37·5	5·42

* The figure for 'ash' has not been corrected for $CaCO_3$.

These figures show that, in the constituents determined, the farmyard manure and the two composts do not differ very widely in their content of dry matter, of organic matter, and of nitrogen, though the compost made with sewage sludge is decidedly the richest in these constituents. The proportion of nitrogen to the organic matter is lowest in the farmyard manure while the composts differ only

a little. The sewage sludge stands on a different footing, with double the amount of organic matter and far more nitrogen in the quantity applied to the land, as well as a higher proportion of nitrogen in the organic matter.

With these materials the results in each of the 3 years for which data have been taken, and for the 3 years taken together, are shown in Table 3.

TABLE 3. *Effect on proportion of bolters of increasing doses of nitrogenous fertilizer (sulphate of ammonia) without any organic manure.*

	Bolters per 1000 plants			
Manurial additions	1946	1948	1950	Mean
No nitrogenous fertilizer	26	16	50	31
4 cwt. sulphate of ammonia per acre	76	63	78	72
8 cwt. sulphate of ammonia per acre	77	98	85	87
12 cwt. sulphate of ammonia per acre	79	99	91	90

The sulphate of ammonia has, in each year, raised very largely the amount of bolting. The increase, when more than 4 cwt. sulphate of ammonia per acre were added, has been small and the change from 8 to 12 cwt. sulphate of ammonia per acre has given a negligible increase.

TABLE 4. *Effect on proportion of bolters of equal quantities of the four organic coarse manures. The figures include all plots, whether they had a further addition of nitrogenous fertilizer or not*

	Bolters per 1000 plants			
Manurial additions	1946	1948	1950	Mean
No organic manure:	51	39	64	52
Farmyard manure:	77	175	168	140
Compost made with dung:	68	160	121	116
Compost made with sewage sludge:	80	108	119	102
Sewage sludge:	153	207	300	220

The effect of the organic manures (Table 4) may be divided into that with the farmyard manure and the composts, and that with sewage sludge. The latter has given more than twice the amount of bolting of the former. Of course it is realized that the actual amount of organic matter and of nitrogen added is, in this case, owing to the relative dryness of the material, more than double that given with the other three materials, and this may account, at least partially, for the vastly increased amount of bolting caused by the sewage sludge. But the difference was very striking in the field, particularly in 1950, and the plots treated with sewage sludge could be picked out by the number of bolted plants on them. The other treatments differ comparatively little—not, in fact, more than might have been expected with such different materials. It is clear, however, that the organic manures, both with and without addition of sulphate of ammonia, have increased the amount of bolting between two and three times (Table 5).

TABLE 5. *Effect on proportion of bolters of increasing quantities of the four organic coarse manures, without any addition of nitrogenous fertilizer*

Manurial additions	Bolters per 1000 plants			
	1946	1948	1950	Mean
No organic manure or nitrogen	26	16	50	31
Farmyard manure:				
15 tons/acre	44	76	93	71
30 tons/acre	98	223	217	179
Compost made with dung:				
15 tons/acre	46	69	61	59
30 tons/acre	65	206	105	135
Compost made with sludge:				
15 tons/acre	79	67	74	74
30 tons/acre	59	113	132	101
Sewage sludge:				
15 tons/acre	128	149	170	149
30 tons/acre	123	271	378	257

The increase of bolting due to the heavier dressing of organic manures is very striking, except in the case of the sludge and sludge compost in 1946, a difference which we are not at present able to explain. This increase is, however, in all cases much less with the sludge materials than with the other organic manures. Doubling the dressing of organic manure has increased the bolting by about $2\frac{1}{2}$ times with farmyard manure and with compost made with dung, while with the sludge materials the increase has only been about $1\frac{1}{2}$ times (Table 5).

TABLE 6. *Effect on the proportion of bolters of the addition of 4 cwt/acre sulphate of ammonia in addition to organic manures*

Manurial additions	Bolters per 1000 plants			
	1946	1948	1950	Mean
Farmyard manure:				
(1) no sulphate of ammonia	76	156	156	129
(2) with sulphate of ammonia	73	201	182	152
Compost made with dung:				
(1) no sulphate of ammonia	56	136	98	97
(2) with sulphate of ammonia	79	175	151	135
Compost made with sludge:				
(1) no sulphate of ammonia	67	91	104	87
(2) with sulphate of ammonia	89	126	142	119
Sewage sludge:				
(1) no sulphate of ammonia	125	203	274	201
(2) with sulphate of ammonia	179	208	340	242

The effect of the addition of 4 cwt. sulphate of ammonia per acre (equal to 84 lb. nitrogen per acre) to the organic manures has differed a good deal with the different organic materials. With the two composts the increase in bolting is between 35 and 40%, with the farmyard manure and the sewage sludge it is far less. In every case,

however, the addition of sulphate of ammonia to the organic manures has definitely increased the amount of bolting.

In 1950 it was possible to make some other observations.

(1) The amount of bolting varies very much with local circumstances. Thus, in the two adjoining blocks in the experiment now being analysed, the total proportion of bolting plants was very different, being nearly double in one block to what it was in the other. The actual mean figures in the two blocks were as follows: Block I, 20·3 %; Block II, 10·9%. Such differences are perhaps to be expected and show that many other factors are involved than the season and the character of the manuring. But the proportion between the increase due to the several treatments was not very widely different. As an illustration, Table 7 gives the increase of bolting due to doubling of the organic manures, both with and without the addition of sulphate of ammonia, taking the amount of bolting with the smaller dressing as

TABLE 7. *Increase of bolting due to doubling of organic manures (amount of bolting with single dose taken as unity).*

	Block I	Block II	Mean
Farmyard manure	2·07	2·94	1·99
Compost made with dung	2·06	2·14	2·08
Compost made with sludge	1·59	1·43	1·53
Sewage sludge	1·94	1·71	1·90

unity. Despite the difference in total bolting in the two blocks, the percentage increase does not differ more widely than would have been expected. Similar results are obtained if other criteria are taken.

(2) An attempt has been made, with the data for the three years, to judge whether anything in the weather conditions during the growth of the beetroot plants would account for the very large amount of bolting particularly in 1948 and 1950. In 1950, the amount of bolting in all sorts of plants which are liable to flower prematurely, in the neighbourhood of the Woburn station, was very large, and many crops of lettuce, for instance, were actually ploughed into the ground as useless on this account. Conditions in the one year (1938) when sugar beet gave a very large percentage of bolters have been given already (p. 437) and the only abnormal condition in that year seemed to be a long drought after sowing which may have caused a check to the early growth. Rainfall in 1946, after the sowing of the beetroot crop, was reasonably normal, but in the two later years the May rainfall was very heavy and it is possible that a check may have been given to the crop by the temporary and partial waterlogging of the soil during this month. Further, the mean temperature during May was low in both years while that in June was higher than normal, leading to a slight check in May followed by specially vigorous growth in June. The possible connexion of these climatic conditions with the proportion of bolting is, however, purely speculative.

REFERENCES

CHROBOCZEK, E. (1934). A study of some ecological factors influencing seed stalk development in beets. *Mem. Cornell agric. Exp. Sta.* no. 154.

HALL, A. D. (1928). Bolting in sugar beet and mangolds. *J. Genetics*, **20**, 219.

WATSON, D. J. & RUSSELL, E. J. (1943). Effect of manures on the growth of the mangold plant. *Emp. J. exp. Agric.* **11**, 65–77.

(*Received* 9 *September* 1950)

RAT POPULATIONS AND CONTROL IN TWO ENGLISH VILLAGES

By S. A. BARNETT,* A. H. BATHARD and MARY M. SPENCER

Ministry of Agriculture and Fisheries Infestation Control Division, Tolworth, Surrey

(With Plates 12 and 13, 9 Text-figures and 2 Maps)

Rat (*Rattus norvegicus* Berkenhout) populations have been studied in two villages in Devonshire. One village had a human population of 266, the other, of 364. The main activity in each village is mixed farming, with poultry and pig keeping on a small scale.

From spring 1947 to spring 1950 visits were made to both villages at six-monthly intervals. At each visit a relative census of the rat populations was taken. This was done either by test baiting, which records only the visits by rats to bait points; or by census baiting, in which a surplus of wheat is laid each day until the amount taken levels off. The level so reached is a measure of the rat population. Test baiting was found to be less reliable, as an index of changes in the rat populations, than census baiting. In some instances treatments, consisting of one, two or three strikes, i.e. poisonings after prebaiting, were carried out after the census.

Complete clearance of a whole village was not achieved on any occasion. Lasting reductions of the rat populations were achieved only by comprehensive double or triple strikes. After such treatments the rat populations took more than a year to recover; probably the recovery to the maximum rat population would often have taken 2 years or more had it been allowed to take place.

In each village the rats were distributed in discrete colonies, most of them in farms or chicken runs. Poor hygiene and dilapidation of buildings were important factors in promoting rat infestation. When a new source of food became available near an existing infestation, e.g. as a result of the setting up of a new chicken run, rats soon appeared.

Apart from rats five species of small mammals visited the bait points: *Mus musculus*, *Apodemus sylvaticus*, *Microtus agrestis*, *Clethrionomys glareolus* and *Sorex araneus*. Their presence was detected by their droppings, by characteristic nibbling of the grain, and by trapping. Allowance was made for their presence in recording the results of census baiting.

Mouse activity at the bait points was greatest when the rat population was low, but there is no certain evidence that mouse numbers greatly increased when the rats were reduced.

It is suggested that, for effective control and the economical use of manpower, treatments in rural areas should always consist of at least two strikes. It may be expected that such treatments, if carried out with a high degree of thoroughness and efficiency, need normally be carried out only once a year; and it might often be possible to leave an area for 18 months or 2 years.

Introduction

In all rat control there is the problem, not only of achieving an immediate clearance, but also of making the clearance lasting. Most of the scientific work on the efficiency of control methods has consisted of operations on small, self-contained infestations,

* Now at Department of Zoology, University of Glasgow.

and has only estimated the degree of clearance obtained immediately after treatment (Barnett, 1948*b*). The standard control methods for towns, based on such studies, involve two or more successive poisonings or *strikes* (each after prebaiting), but in the country the procedure is different: the usual practice is 3-monthly visits to sites, such as farms, liable to infestation. A single strike is carried out at each visit. This procedure is convenient, but no study of the effect on the rat populations has been made. The only objective data on the rural rat population of England are given in Elton's (unpublished) inquiry on the rat populations of ricks. This inquiry covered the years 1942–6, and indicated that little progress was made in reducing the rick rat population during that time. Middleton (unpublished) describes the successful control of the rat population of a group of farms at small cost, but the area he studied contained no villages.

The problem can be put in another form. In any area at any time there is a given labour force available for rodent control. The procedure employed may be that of a single strike at 3-monthly intervals; it may be systematic block clearance on the lines officially laid down for towns (Barnett, 1946); it may be no more than single poisonings in infested premises in response to complaints made by occupiers. We have no data to help us to decide which set of practices is economically best, and to get such data is exceedingly difficult. The present inquiry is a first attempt to tackle this problem for village rats (*Rattus norvegicus* Berkenhout).

The villages

(*a*) *General*

The two villages studied, which we shall call B and S, are both in south Devonshire. S had 364 people, 55 cats and 25 domestic dogs; for B, the corresponding figures were 266, 43 and 14. Both villages contain farm buildings and are isolated by farmland from other built-up areas or farms (maps 1 and 2). Moreover, there were no ricks within or near the villages; mixed farming is the rule, with chicken and pig keeping in backyards. Consequently the rat population of each village can be regarded as self-contained: inspection failed to show any trace of rats in the surrounding fields or hedges.

The small dwellings and farm buildings which make up a great part of each village have mud and rubble (cob) walls and often thatched roofs. Others are built of dry stone. Both types of structure offer plenty of harbourage for rats (Pl. 12). Chicken runs, farmyards and hedgerows also provide harbourage (Pl. 13). Buildings for the most part are old and often in bad repair. Hygiene in the farms is generally poor, but variable.

There are small sewer systems in each village, but neither was infested with rats. S has a sewage farm half a mile outside the village and this was at one time slightly infested; it was, however, not of importance for the inquiry, and it is not included in the results given below.


VILLAGE. B.
100 0 100 200 300 400 500
FEET
N
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15

Map 1.

Map 2.

(*b*) *Previous history of rat control*

Special attention was first paid to the rat population of these villages in 1945. A 36 sq. mile area in which they lie had been used by troops for battle practice during the war, and the human population had been evicted. When the inhabitants returned, in October 1944, they found a large rat population which had evidently been living on the waste food left by the troops. For a short period there had been neither people nor livestock in the area, and probably by that time the rats were short of food: they were observed to strip bark from trees and to eat the putty from newly repaired windows.

During the period January to March 1945, systematic rat control was carried out in the whole area, with its nine villages and many isolated farms. Each village received two strikes, and the results of the baiting suggested that a high degree of clearance was achieved (Table 3).

During the rest of 1945 the only rat control in the villages was carried out by the Rural District Council in response to a few complaints by occupiers. In 1946, under a general direction issued by the Ministry of Food to all local authorities, the Council attempted a systematic treatment of each village, and the operator concluded that two of the villages had especially troublesome rat populations: these were B and S. It was therefore thought that an inquiry into the state of the rat population in these villages might yield useful results, and from spring 1947 to spring 1950 a series of operations was carried out in them.

Methods

(*a*) *Summary of methods*

Throughout the inquiry visits were made at 6-monthly intervals, in spring and autumn. At each visit the rat population was estimated either by a 'test baiting' or a 'census baiting'. On certain visits poison treatments were also given, immediately after the test or census. The sequence of events for each village is shown in Tables 1 and 2.

(*b*) *Census methods*

The chief problem was the estimation of changes in the rat populations. In the early stages an attempt was made to do this by test baiting. Bait points were selected in all places which were, or might be, infested. The bait was laid in containers (Pl. 12, fig. 2; Pl. 13, fig. 4) or occasionally (in the buildings) on flat wood trays. 30 g. bait was laid at each point, and the point revisited after 2 days. It was then recorded whether all, some or none of the bait had gone.

After three visits, an attempt was made to get a more accurate relative census of the rat populations by using Chitty's method (Chitty, 1942; Chitty & Shorten, 1946). This involves laying a surplus of plain dry wheat, at bait points as already described, and weighing the residues each day, to the nearest 10 g., until the daily take is steady. Text-figs. 1–5 show the amounts of wheat eaten each day during each census.

TABLE 1. *Time-table of operations: village B*

Date	Operation	Materials
1945		
9–12 Jan.	Treatment	Base: damp rusk
13 Jan.		Poison: red squill (10%)
15–18 Jan.		Base: bread mash
19 Jan.		Poison: arsenious oxide (10%)
Later in year	Two isolated treatments by Local Authority in response to complaints	Not known
1946		
Aug.	Single strike by Local Authority	Not known
1947		
17–20 June	Treatment	Base: damp rusk
21 June		Poison: zinc phosphide (2½%)
8–11 July		Base: flour+sugar (10%)
12 July		Poison: arsenious oxide (15%)
6–8 Oct.	Test bait	Dry wheat
1948		
5–7 Apr.	Test bait	Soaked wheat
27–29 Sept.	Test bait	Soaked wheat
5–8 Oct.	Treatment	Base: damp rusk
9 Oct.		Poison: zinc phosphide (2½%)
1949		
25–27 Apr.	Test bait	Soaked wheat
28 Apr.–6 May	Census 1	Dry wheat
10–13 May	Treatment	Base: damp rusk
14 May		Poison: zinc phosphide (2½%)
31 May–3 June		Base: soaked barley
4 June		Poison: antu (2%)
21–24 June		Base: flour+sugar (10%)
25 June		Poison: arsenious oxide (15%)
12–15 July		Base: damp rusk (site 13 only)
21 Sept.–1 Oct.	Census 2	Dry wheat
1950		
13–21 Apr.	Census 3	Dry wheat

Most censuses went on for 9 days, but two for 11 days. In all censuses taken together, the percentage of points visited by the rats for the first time, by day of census, was as follows:

Day.....	1	2	3	4	5	6	7	8
Village B	44	24	12	12	6	0·6	1·2	0
Village S	44	21	16	9	6	2	0·5	0·5

The number of bait points in B varied from 115 to 146, and in S from 77 to 143.

It was assumed at first that only rats would enter the containers and eat the bait, but during the test baiting in October 1947 the appearance of the bait at many points suggested that mice and not rats were visiting it. In some places mouse droppings were seen. It is usually possible to distinguish mouse kibble from rat kibble (Spencer, unpublished), and where only mouse kibble or droppings were observed during

Table 2. *Time-table of operations: village S*

Date	Operation	Materials
1945		
13–16 Feb.	Treatment	Base: damp rusk
17 Feb.		Poison: red squill (10%)
20 Feb.	Post bait	Base: flaked wheat
Later in year	Four isolated treatments by Local Authority in response to complaints	Not known
1946		
Aug.	Single strike by Local Authority	Not known
1947		
29 Apr.–2 May	Treatment	Base: damp rusk
3 May		Poison: zinc phosphide (2½%)
3–6 June		Base: flour+sugar (10%)
7 June		Poison: arsenious oxide (15%)
8–10 Oct.	Test bait	Dry wheat
1948		
5–7 Apr.	Test bait	Soaked wheat
27–29 Sept.	Test bait	Soaked wheat
30 Sept.–8 Oct.	Census 1	Dry wheat
12–15 Oct.	Treatment	Base: damp rusk
16 Oct.		Poison: zinc phosphide (2½%)
2–5 Nov.		Base: soaked barley
6 Nov.		Poison: antu (2%)
23–26 Nov.		Base: flour+sugar (10%)
27 Nov.		Poison: arsenious oxide (15%)
1949		
25–27 Apr.	Test bait	Soaked wheat
28 Apr.–6 May	Census 2*a*	Dry wheat
10–18 May	Census 2*b*	Dry wheat
21 Sept.–1 Oct.	Census 3	Dry wheat
4–7 Oct.	Treatment	Base: damp rusk
8 Oct.		Poison: zinc phosphide (2½%)
25–28 Oct.		Base: soaked barley
29 Oct.		Poison: antu (2%)
30 Oct.–3 Nov.	Census 4	Dry wheat
28 Nov.–1 Dec.	Isolated treatment by Local Authority	Base: flour+sugar (10%)
2 Dec.		Poison: arsenious oxide (15%)
1950		
16–19 Jan.	Isolated treatment by Local Authority	Base: rusk, flour+sugar
20 Jan.		Poison: zinc phosphide (3%)
6–9 Mar.	Isolated treatment by Local Authority	Base: rusk, flour+sugar
10 Mar.		Poison: zinc phosphide (3%)
13–21 Apr.	Census 5	Dry wheat

a census the point was recorded as having been visited by mice and not rats. Mouse traps, usually of the breakback type, were set after censuses or treatments where mice were suspected, so that the species could be identified.

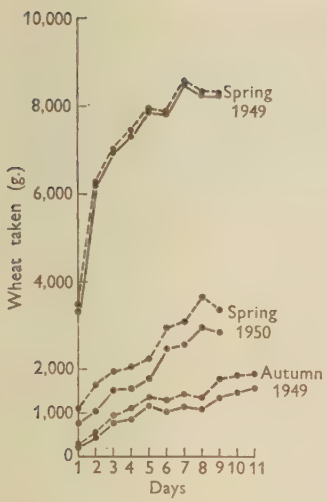


Fig. 1.

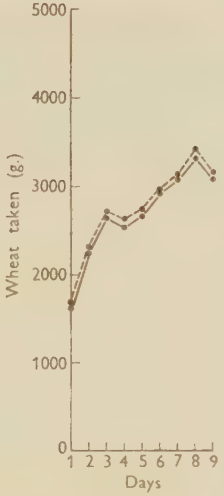


Fig. 2.

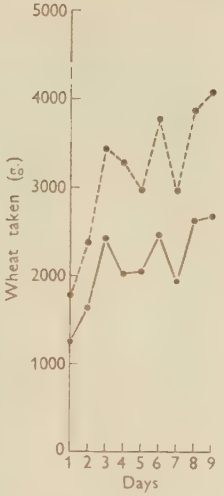


Fig. 3.

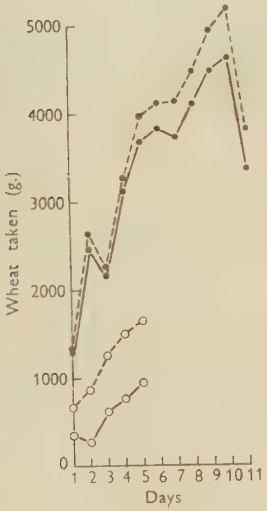


Fig. 4.

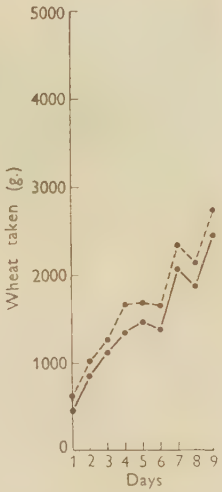


Fig. 5.

Text-fig. 1. Village B. Censuses. Total, •- - - -•; *R. norvegicus* only, •———•.

Text-fig. 2. Village S. Census Autumn 1948. Total, •- - - -•; *R. norvegicus* only, •———•.

Text-fig. 3. Village S. Census Spring 1949. Total, •- - - -•; *R. norvegicus* only, •———•.

Text-fig. 4. Village S. Censuses Autumn 1949. 21 Sept.–1 Oct. First census. Total, •- - - -•; *R. norvegicus* only, •———•. 30 Oct.–3 Nov. Second census (after treatment). Total, ○- - - -○; *R. norvegicus* only, ○———○.

Text-fig. 5. Village S. Census Spring 1950. Total, •- - -•; *R. norvegicus* only, • —•.

(*c*) *Methods of treatment*

The methods of poisoning were those officially recommended (Barnett, 1946). The materials used are shown in Tables 1 and 2. In each strike baits were laid in containers, in holes or on trays. The containers, which prevented access by farm animals, were always in place for some weeks before baiting began. For 4 days plain bait was laid, and on the fifth day the same bait base with poison added. Second strikes, with a different bait base and poison, were made a fortnight after the first. When there was a third strike this was done with a third base and a third poison a fortnight after the second.

Some minor rat control after the main treatments was carried out by means of breakback traps. In addition, during the whole period, a certain amount of unprompted control was done by the human population and dogs and cats. Methods included shooting, trapping and poisoning with proprietary baits. As will be seen later, this did not prevent the rats from multiplying after their numbers had been reduced by poison treatments.

(*d*) *Discussion of methods*

No method exists for the accurate estimation of the *absolute* number of rats in an area such as a village. The two methods described above, test baiting and census baiting, were intended to provide indices of the *relative* numbers of rats (cf. Emlen, Stokes & Davis, 1949; Chitty, unpublished).

In a census the consumption of alternative food is avoided as far as possible by placing the bait points so near to the nesting sites that the rats are intercepted on their way out. In places such as grain stores, however, it is hardly likely that this will prevent them from taking some of their food elsewhere. In general, the proportion of alternative food eaten may vary both with the place and with time. This must tend to give rise to an error in the census figures, and we have no means of estimating its magnitude. In a whole village, however, it is probable that such effects are less than they would be in a small area such as a single farm.

A further question is the extent to which test baiting gave valid information. In B the results of test baiting (Text-fig. 6) were consistent with those of census baiting (Text-fig. 7). In S, however, this was not the case (Text-figs. 8 and 9). It seems likely that in S conditions were such that the number of rats could vary widely without greatly influencing the *number* of points visited during a test bait; the variation was, however, reflected in the *quantity* of wheat eaten during census baiting. In B, on the other hand, an increase in the rat population was evidently accompanied by a spread over a wider area, and this was reflected in the number of points visited. This is no more than hypothesis, but we can conclude that, as might be expected, test baiting is less reliable than census baiting.

A third possible method of estimating rat populations is by means of traces: holes, runs, droppings, damage and other clues to the presence of rats. It has been claimed

that in urban (though not rural) areas the study of traces can give reliable estimates of relative numbers (Emlen, *et al.* 1949). We do not think that such a method could be applied in the conditions of these villages. Traces of rat activity depend on many variable factors, including the amount of cover, the amount of waste food scattered about and how hungry the rats are. We found many examples of discrepancies between traces and the rat population indicated by census baiting.

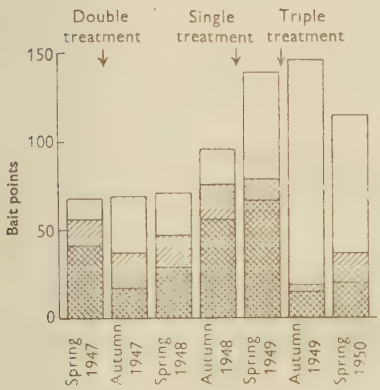


Fig. 6.

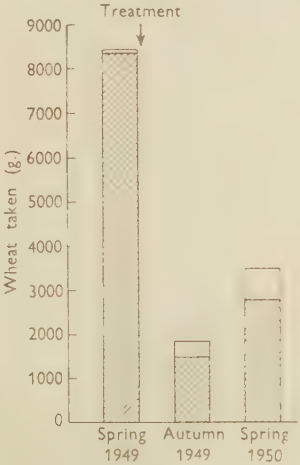


Fig. 7.

Text-fig. 6. Test baiting. Village B. Points laid, □; *R. norvegicus* only—partial takes, ▨; complete takes, ▩.

Text-fig. 7. Census averages. Village B. Rats, ▨; mice, □.

In the description of the results it is assumed that the figures derived from census baiting give an accurate index of the rat populations. The conclusions drawn from the results of test baiting are tentative, and where the results of test baiting are inconsistent with those of census baiting, they are assumed to be misleading.

Results

(*a*) *Poison treatments*

The records of poison treatments are given in Tables 3 and 4. The marked decline in the number of points visited in second strikes is characteristic of operations carried out on standard lines. On the occasions when third strikes were made the amount of bait taken was in most places negligible. The decline in take in second and third strikes is sometimes thought to indicate the degree of success of the poisoning, but it is doubtful whether this assumption is justified. Only a census, carried out

at different points from those used in poisoning, can give a reliable estimate of the residual rats.

Other observations arising from poison treatments are described below.

Table 3. *Summary of treatments: village B*

Date	No. of bait points	Prebait takes	Poison takes
9–13 Jan. 1945	508	332	304
15–19 Jan. 1945	68	49	18
17–21 June 1947	68	59	45
8–12 July 1947	57	16	12
5–9 Oct. 1948	106	102	78
10–14 May 1949	109	104	103
31 May–4 June 1949	137	71	64
21–25 June 1949	66	45	44
12–15 July 1949	20	0	—

Table 4. *Summary of treatments: village S*

Date	No. of bait points	Prebait takes	Poison takes
13–17 Feb. 1945	401	278	273
20 Feb. 1945	117	0	—
29 Apr.–3 May 1947	138	117	89
3–7 June 1947	37	32	27
12–16 Oct. 1948	97	95	77
2–6 Nov. 1948	89	50	34
23–27 Nov. 1948	52	36	22
4–8 Oct. 1949	77	70	60
25–29 Oct. 1949	114	76	52

(*b*) *Effects of treatments on rat populations*

(i) *Village B.* Text-fig. 6 shows the results of test baiting in B. It includes the results of the first 2 days' prebaiting in spring 1947, and of the first 2 days' census in spring 1950, since both operations provide an equivalent to test baiting as described above.

Immediately after the test baiting in spring 1947, an attempt was made to clear B of rats by the standard method of two strikes. Six months later test baiting was repeated. The result of this test, taking place after a period during which breeding was presumably at a high rate, suggested that the spring clearance must have been successful: thirty-seven points, instead of fifty-six, were visited by rats, and the bait was completely taken at only seventeen. In spring 1948 a third test suggested that the degree of infestation was nearly back to what it had been a year earlier, and by autumn 1948 the infestation recorded was higher than that of spring 1947.

After the autumn test in 1948 a single strike was carried out. This operation was done as thoroughly as possible, but omitted one farm (site 13, map 1) owing to the opposition of the occupier. Six months later, in spring 1949, the degree of infestation indicated by test baiting was almost exactly the same as it had been in autumn 1948.

The single strike might never have taken place. On this occasion a full census lasting 9 days was also carried out, and the mean daily take for the last 3 days was 8400 g. (Text-fig. 7).

Immediately after the census a full-scale treatment, with three strikes, was carried out; this included the farm which had previously been omitted. Six months later, in autumn 1949, both test and census baiting indicated the presence of a relatively small rat population. The census figure (for rats only) was 1400 g. or 17% of the spring census value despite the intervening breeding season. The number of points visited during the autumn test baiting was 24% of the spring figure. Finally, in spring 1950, a further census was taken, and this showed that the infestation was still only 33% of that of a year before.

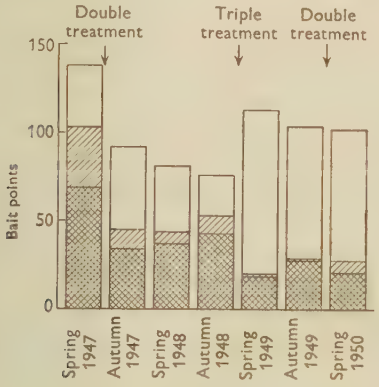


Fig. 8.

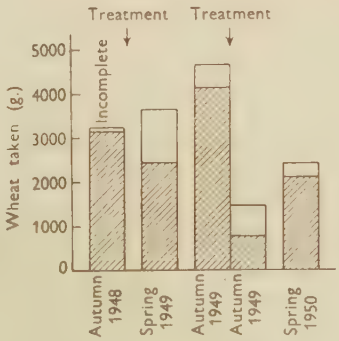


Fig. 9.

Text-fig. 8. Test baiting. Village S. Points laid, □; *R. norvegicus* only—partial takes, ▨; complete takes, ▩.

Text-fig. 9. Census averages. Village S. Rats, ▨; mice, □.

(ii) *Village S.* Text-fig. 8 shows the results of test baiting in S. They suggest that, as in B, the degree of clearance achieved in spring 1947 was high, since less than half of the points visited in spring 1947 were visited in the autumn. The following spring there was little further rise recorded; this might be attributed to the absence of breeding during the winter. However, in autumn 1948 there was still only a slight further rise, and it is possible that the increase shown by test baiting failed to reflect the increase in rat numbers. From autumn 1948 census baiting was done, and we can consider the evidence which it provides (Text-fig. 9). In autumn 1948, 18 months after the previous treatment, the census figure was 3200 g. This census was not quite complete, since one farm (site 10, map 2) was omitted owing to the opposition of the occupier; it was learnt later that two chicken runs were also omitted (site 1). This census was followed by a full treatment of three strikes, but this too omitted the two sites mentioned. Six months later, in spring 1949, the

census figure for rats was 2400 g. This census was, however, complete, since the two sites omitted in autumn 1948 were now included. Proportionally, therefore, the reduction in population must have been greater than that shown. In autumn 1949 a further complete census gave a figure of 4200 g. The test-baiting figures for this period, and especially for autumn 1949, do not conform with the census figures.

After the first census in autumn 1949 a full treatment was given, and was immediately followed by a further census. This census, although it was carried on for only 5 days, gave the surprisingly high figure of 800 g., which suggests that the degree of clearance (only 81%) was less than had been expected. In spring 1950 the census figure for S was 2100 g., slightly below that of a year before but still only 66% of the incomplete figure for autumn 1948.

(*c*) *Sites of infestation*

(i) *Village B.* The main habitats of the rats were farm buildings and yards, and poultry runs. There was no simple correlation between the size of an infested area and the rat population. For example, site 10 in B (map 1) consisted of a number of farm buildings, a yard, a joiner's shop and a chicken run, covering an area of 7800 sq.ft. Site 11 was simply a small chicken run established along a hedge bank on the other side of which was rough pasture, and its area was only 400 sq.ft. Yet site 11 in spring 1949 gave a census figure almost exactly the same as that of site 10, and in later censuses the figure for site 11 was the higher.

The detailed records for spring 1949, compared with those for spring 1950, show that four sites in particular had a considerable infestation. Sites 10 and 11 have been described; the other two, sites 13 and 14, were both areas of farm buildings and included chicken runs. Site 13 was the one omitted from the census of spring 1949. The persistent infestation in these places was obviously related to poor hygiene and to the quantities of food accessible to the rats.

(ii) *Village S.* The situation in S (map 2) was, in general, similar to that in B. Again farms and chicken runs were the main sites of infestation, and again the area of the site was no guide to the degree of infestation.

In some places new infestations arose as a result of changes in the environment. Site 16 (map 2) is an example: here farm buildings and a chicken run on the east side of the road were infested, but the hedge bank and field immediately opposite were clear. During the winter of 1949–50 chickens were installed in the field on the west side of the road, and during the census of spring 1950 rats were detected there for the first time.

(*d*) *Effects of treatments on mouse populations*

(i) *Extent of infestation.* In B signs of mice were chiefly observed in hay lofts and grain or feed stores, but in S, where mice were more obtrusive, they were noticeable also in hedge banks within the village. In both villages the amount of census bait taken by mice tended to be higher when the rat population was low.

TABLE 5. *Mice trapped: village B*

Date	Site	Species			
		Mus	*Apodemus*	*Microtus*	*Clethrionomys*
Oct. 1947	7: hedge (traps also set at other sites)	—	2	—	—
Apr. 1948	10: grainstore (traps also set at sites 15 and 7)	2	12	—	—
Dec. 1948	10: grainstore	8	—	—	—
May 1949	10: grainstore	4	—	1 (poisoned)	—
	7: outbuildings and hedge	2	1	—	—
Apr. 1950	10: tractor shed	1	—	—	—
	8: chicken house, rubbish tip	2	—	—	1
	13: hay loft	1	—	—	—
	2: byre	1	—	—	—
	2: food store	1	—	—	—

TABLE 6. *Mice trapped: village S*

Date	Site	Species				
		Mus	*Apodemus*	*Microtus*	*Clethrionomys*	*Sorex*
Oct. 1947	Other: garden (traps also set at other sites)	1	2	—	—	—
Apr. 1948	—	—	—	—	—	—
Dec. 1948	15: hedge (no traps set elsewhere)	—	1	—	—	—
May 1949	9: loft	3	—	—	—	—
	11: hedge	16	1	8	—	3
	13: outbuilding	3	—	—	—	—
	14: loft	2	—	-	—	—
	15: hedge	5	5	1	—	—
Nov. 1949	1: hedge	—	—	1	—	—
	3: garden	—	6	1	—	—
	6: barn	—	—	1	—	—
	12: outbuilding	1	—	—	—	—
	16: barns	3	—	—	—	—
Apr. 1950	3: garden	—	5	—	1	—
	9: barn	4	—	—	—	—
	11: hedge	1	—	—	2	—
	16: hedge	2	—	—	—	—
	Other: garden	—	1	—	—	—

In B the figures for mice in spring and autumn 1949 may be compared (Text-fig. 7; Table 5); there is a marked increase, accompanying the reduction in the rat population. The further increase in mouse activity recorded in spring 1950, when the rat population was higher, is due to sites 2 and 13, and neither of these was baited in spring 1949. S provides clearer evidence of the relation between rat numbers and mouse activity at the bait points (Text-fig. 9; Table 6).

(ii) *The mouse species.* Four species of small rodent were trapped at bait points: *Mus musculus* L., the house mouse; *Apodemus sylvaticus* L., the long-tailed field mouse; *Microtus agrestis* L., the short-tailed field vole; and *Clethrionomys glareolus*

Schroeber, the bank vole or red mouse. In both villages more house mice were caught than any other species; after these came *Apodemus*. The other two species were evidently of little importance (Tables 5 and 6). In addition to the rodents three *Sorex araneus* L., the common shrew, were trapped in spring 1949, at site 11 in S.

The trapping results were designed to give information only on what species were present, and not on their numbers: to achieve even a relative census of these small mammals would require a much more elaborate trapping programme.

Discussion

(*a*) *The changes in the rat populations*

If the habitats provided by the villages are regarded as constant over the period of the investigation, it may be assumed that the rat populations after reduction by poisoning would increase at a declining rate to an asymptote representing the maximum capacity of the area. Unfortunately our estimates of the relative size of the rat populations were too infrequent to give any information on the shape of their growth curves.

It is however possible to suggest some tentative conclusions on the effectiveness of the different grades of treatment. In B the shortest treatment was the imperfect single strike in autumn 1948, and we have seen that 6 months later no effect of this operation could be detected (Text-fig. 6). On the other hand, the effect of the incomplete double treatment in spring 1947 was detectable (by test baiting) a year later; and a year after the complete triple treatment in spring 1949 the rat population was still low (Text-fig. 7).

In S the picture is not quite so clear. It is probable that the rat population never fully recovered from the double treatment of spring 1947; this is at least suggested by the results of the test baiting (Text-fig. 8). It is unfortunate that no census baiting was done in spring 1947, and that consequently the only comparison that can be made is of the test baiting figures. The effects of the triple treatment in autumn 1948 are masked by the fact that the first census after this treatment included the two sites which had previously been omitted. Apart from this, the general picture is similar to that shown by B (Text-fig. 9).

It might be thought that thorough treatments should be capable of achieving complete clearance of small, isolated communities such as these villages. Every effort was made to make the treatments as efficient and comprehensive as possible, but the proportion of rats killed always fell short of 100%. The sites with the most persistent infestations—the farms and chicken runs in paricular—were all conspicuous for defective hygiene and for the dilapidation of the buildings. This conforms with the observation of Davis & Fales (1949) that in urban areas there is a positive correlation between the density of the rat population and the proportion of dilapidated buildings. Probably, however, dilapidation is less important in a rural than an urban area, since in the country there is usually earth in which the

rats can burrow: they are not obliged to rely wholly on man-made cover. Consequently, the limiting factor may often be the availability of food. This was exemplified by the rapid appearance of rats in association with new chicken runs near existing infestations, with their inevitable scatter of chicken feed. There is little doubt that in some areas the keeping of backyard poultry and small-scale poultry farming plays a major part in maintaining the rat population (Kirby, 1945).

An important practical question is the extent to which communities such as S and B can be regarded as single units. Examination of the maps suggests that the rats in each village were distributed in discrete colonies. What knowledge there is of the ranging habits of the common rat suggests that these colonies themselves can reasonably be regarded as isolated, at least for periods of some months. Davis, Emlen & Stokes (1948) report only a very small range of normal movement in *Rattus norvegicus* both in Baltimore and on a farm: in Baltimore, when rats were trapped, marked and released, about 80% were recaptured within 60 ft. of the point of the original capture; similarly, the distribution of coloured dung round bait stations in which food containing a dye had been laid indicated a range of about 100 ft. in diameter.

Our own observations provide only indirect evidence on this question. When, in autumn 1948, S was given a triple treatment omitting two sites, it was thought that it might be possible to trace a spread of re-infestation from the omitted farm (site 10), but no such effect was observed. Certain sites in both villages were completely cleared by treatments, and then, as would be expected of isolated sites, remained clear or almost clear for 12–18 months. Emlen, Stokes & Winsor (1948) give an example of the effects of complete clearance of a city block: rats did not re-appear until the thirtieth month after the clearance, and in the thirty-eighth month the population was estimated still to be less than 50% of what it had been before treatment. It is, however, unlikely that any of the infested sites in S or B would remain clear for so long, since the barriers to rat movement in a village are probably less effective than those in a city (cf. Emlen, Stokes & Davis, 1949).

The complete clearance of some sites has a bearing on the rate at which the village rat populations as a whole are restored. The sites which have been completely cleared make no contribution, at least for a long time, to the rebuilding of the population, and so the population growth rate is likely to be slower than it would be if every site were given the same, incomplete degree of clearance.

All these points together suggest the following provisional conclusions. After a comprehensive and efficient treatment of two or three strikes the rat population of areas such as B and S is unlikely to be restored to its maximum in a year; it might well be that the maximum would often not be reached even after 2 years. On the other hand, at a few sites rats might become a nuisance again comparatively rapidly, probably within 12 months. A similar conclusion was reached when a very thorough anti-rat campaign had been carried out after a plague outbreak in Malta in 1945–7 (Barnett, 1948*a*).

The invasion of new sites must depend partly on the population pressure in neighbouring infested areas. Our observations suggest however that it is not necessary for an area to have a rat population even near the maximum it can support before it gives rise to new colonies. Calhoun's (1949) study of intraspecific competition in *R. norvegicus* gives a possible explanation of this fact.

(*b*) *The absolute numbers of rats*

No attempt was made in this investigation to estimate absolute numbers of rats, but our data give some evidence on this point. The average daily consumption of whole wheat by *R. norvegicus* eating no other calorigenic food is approximately 24 g. (Leslie, unpublished). The results of our censuses therefore provide minimum figures for the rat populations of the two villages. On this basis the rat population of B in spring 1949 was at least 330, rather more than the human population. The rat population of S in autumn 1948 was not less than 180. However, even if all the rats in both villages were attracted to the bait points (which is uncertain), it is probable that the actual rat populations were far higher. Emlen, *et al.* (1949) believe that while a very high proportion of a rat's food is often derived from the bait points during a census, where there is much alternative food the proportion of census bait eaten may fall to 50% of the total (see Chitty, unpublished, for a full discussion). Since there was much alternative food in most of the sites in both S and B, it is likely that the actual rat populations were much higher than the minimum figures given.

(*c*) *The mouse populations*

Although at first sight our results might suggest that reduction in rat numbers was regularly followed by a marked increase in the *numbers* of mice, especially the house mouse and the long-tailed field mouse, all that can safely be said is that mouse *activity* at the bait points increased with the disappearance of the rats. The home range of mice is much smaller than that of rats (Evans, 1942; Southern, unpublished), and bait points numerous enough to cover a rat population would be too few to cover a mouse population in the same area. Moreover, the main habitats of three of the four species of small rodent must have been fields, woods or hedgerows where there were no rats; and even the house mouse may live in fields away from buildings and ricks (Southern & Laurie, 1946). There may consequently have been little increase in the mouse population as a whole, but only a slight local increase as the mice entered places left vacant by the rats.

The mouse spread seems to take place rapidly. In S in 1949 the poisoning must have accounted for many of the mice feeding at the bait points, yet the amount of bait taken by mice in the census which followed the treatment was greater than in the census which preceded it (Text-fig. 9).

The general question is: to what extent is there competition for food or shelter between the common rat on the one hand and the various smaller species on the

other? No doubt there is such competition, perhaps especially with the house mouse, which, like rats, is a regular inhabitant of buildings. Fenyuk (1941) considers that *R. norvegicus* colonizes ricks partly to eat *Apodemus* and *Clethrionomys*. The problem of the relationship between rat and mouse numbers is a subject requiring further inquiry.

(*d*) *Economic implications*

One of the aims of rat control is to reduce the population to so low a level that no further work is needed for a considerable period. If the population recovers within a few weeks the effort expended on the control is wasted. How long the intervals between necessary treatments must be, to be economically acceptable, may depend on the labour force available or on the cost of rat infestation in relation to the cost of control (cf. Barnett, 1948*a*,*b*). The cost of control will in turn depend on whether continued rat control after an initial knock-down of the population can be carried on at a relatively low level of effort. In Middleton's (unpublished) work on a group of English farms the necessary expenditure on maintenance control was found to be relatively low. This however is not always the case, and might not be the case for villages such as those described here. On the subject of costs of rat infestation few exact data exist; Elton (unpublished) gives some estimates of damage by rick rats, but little else of this sort has been attempted. It is therefore necessary to fall back on *a priori* considerations in determining what control programme is economically worth while.

The most important principle is that which suggests the probable form of the growth curve of the rat population. In a constant environment, the growth curve may be assumed to be a logistic. As Davis (1948) has pointed out, most rat destruction reduces the rat population only to a point on this curve at which rate of increase is at or near the maximum: the population is therefore rapidly restored, just as was that of B after the single strike in autumn 1948. If, however, a rat population is reduced to a very low level, say to 1 or 2% of its maximum, its growth rate will for some time be relatively low.

This principle, if it can be applied, is of great practical importance. In terms of cost per rat killed it is much more expensive to reduce a population numbering 10% of the maximum to 1%, than to reduce it from 100% to 10%; yet by the creation of a much more lasting effect such a further reduction may be economically fully worth while or indeed essential.

An obvious conclusion from our work is that even with experienced workers it is very difficult to get a lasting reduction in village rat populations. Control was, however, successful in bringing the rat population of both B and S so low that on two occasions the population 12 months later was still much below the original level, and this suggests that these populations, taken as a whole, were reduced well below the level of maximum growth rate. This degree of efficiency must clearly be the aim in all rat control work. If this is impracticable, comprehensive systematic control operations may be economically not worth while.

These conclusions are tentative, and further operational research is required. Other urgently needed research is on methods which might lead to easier control techniques. One of the main difficulties of large-scale control by present methods arises from the necessity for the elaborate procedures of prebaiting and change of bait and poison (cf. Armour & Barnett, 1950). If they could be made unnecessary a great advance in control efficiency might be rapidly achieved. In the long run it is possibly true, as Davis (1948) suggests, that finally satisfactory rat control will be found to depend to a considerable extent on general improvement of hygiene and building construction. We are, however, such a long way from any widespread improvement on these lines that it is justifiable to exert a great deal of effort on methods of direct rat destruction.

We are grateful to the Rural District Council concerned for permission to work in their district, and for much help given during the course of the inquiry. Acknowledgement is due also to Mrs B. M. Izmidlian who did much of the field work in the early stages, to H. Bradford and W. S. Lawson who helped with the field work throughout, and to A. G. Jenson, F.R.I.B.A., for the maps and diagrams.

REFERENCES

Armour, C. J. & Barnett, S. A. (1950). The action of dicoumarol on laboratory and wild rats, and its effect on feeding behaviour. *J. Hyg., Camb.*, **48**, 158.

Barnett, S. A. (1946). *Infestation control: rats and mice.* London: H.M.S.O.

Barnett, S. A. (1948*a*). Rat control in a plague outbreak in Malta. *J. Hyg., Camb.*, **46**, 10.

Barnett, S. A. (1948*b*). Principles of rodent control. In *Preservation of grains in storage.* (F.A.O. Agric. Publ. no. 2.) Washington.

Calhoun, J. B. (1949). A method for self-control of population growth among mammals living in the wild. *Science*, **109**, 333.

Chitty, D. (1942). Relative census method for *Rattus norvegicus*. *Nature, Lond.*, **150**, 59.

Chitty, D. (unpublished). *Bureau of Animal Population reports.*

Chitty, D. & Shorten, M. (1946). Techniques for the study of the Norway rat (*Rattus norvegicus*). *J. Mammal.* **27**, 63.

Davis, D. E. (1948). Principles of rat management. *Pests*, November, p. 9.

Davis, D. E., Emlen, J. T. & Stokes, A. W. (1948). Studies on home range in the Brown rat. *J. Mammal.* **29**, 207.

Davis, D. E. & Fales, W. T. (1949). The distribution of rats in Baltimore, Maryland. *Amer. J. Hyg.* **49**, 247.

Elton, C. (unpublished). *Bureau of Animal Population reports.*

Emlen, J. T., Stokes, A. W. & Davis, D. E. (1949). Estimating populations of brown rats in urban habitats. *Ecology*, **30**, 430.

Emlen, J. T., Stokes, A. W. & Winsor, C. P. (1948). The rate of recovery of decimated populations of brown rats in nature. *Ecology*, **29**, 133.

Evans, P. C. (1942). Studies of a small mammal population in Bagley Wood, Berkshire. *J. Anim. Ecol.* **11**, 150.

Fenyuk, B. K. (1941). In Symposium *Rodents and rodent control*, no. 1.*

Kirby, G. D. (1945). Rat destruction work in Norwich. *J. R. San. Inst.* **65**, 160.

* Read in translation at the Bureau of Animal Population, Oxford.

Fig. 1

Fig. 2

BARNETT, BATHARD AND SPENCER—*Rat populations and control in two English villages*

Fig. 3

Fig. 4

Leslie, P. H. (unpublished). *Bureau of Animal Population reports.*
Middleton, A. D. (unpublished). *Bureau of Animal Population reports.*
Southern, H. N. (unpublished). *Bureau of Animal Population reports.*
Southern, H. N. & Laurie, E. M. O. (1946). The house-mouse (*Mus musculus*) in corn ricks. *J. Anim. Ecol.* **15**, 134.
Spencer, M. M. (unpublished). Observations on the behaviour of rats and mice feeding on whole grains.

EXPLANATION OF PLATES 12 AND 13

Plate 12

Fig. 1 Cattle shelter in S (site 16).
Fig. 2 'P3' bait container in chicken run in S (site 11).

Plate 13

Fig. 3 Poultry yard at site 11 in S with pigsty on right.
Fig. 4 Weighing wheat from drainpipe containers in chicken run in S (site 1).

(*Received* 15 *July* 1950)

CONTROL OF THE EUROPEAN RABBIT (*ORYCTOLAGUS C. CUNICULUS* L.)

AN EXPERIMENT TO COMPARE THE EFFICIENCY OF GIN TRAPPING, FERRETING AND CYANIDE GASSING

By HARRY V. THOMPSON and C. J. ARMOUR

Ministry of Agriculture and Fisheries Infestation Control Division, Hook Rise, Tolworth, Surrey

(With 3 Text-figures)

Two similar adjacent areas of grass-heath were enclosed by rabbit-proof netting and the bulk of the rabbits on one area removed by gin trapping (in the holes) and on the other by ferreting. Trapping was followed by pump gassing and ferreting in its turn by spoon gassing, with a cyanogenetic powder.

Gin trapping yielded a higher return of rabbits per man hour (1·5) than ferreting (1·0), particularly during the initial stages of clearance. Ferreting gave a steady return.

Pump gassing of burrows, when surface-living rabbits were present, proved ineffective, owing to re-opening of holes from the outside.

Spoon gassing of burrows, integrated with the removal of surface-living rabbits by 'dog and gun', was successful but expensive in time (0·5 rabbits per man hour), compared with gin trapping and ferreting.

The valuable place of 'dog and gun' in planned rabbit clearance was clearly demonstrated.

Trapping yielded equal numbers of bucks and does. Ferreting yielded a higher proportion of does. Spoon gassing (after ferreting) yielded more bucks than does when the holes were dug out. Surface-living rabbits were predominantly bucks.

It is difficult to compare methods of control of any mammal under natural conditions, and not least in the case of the rabbit. The obvious approach is to take two physically similar areas, harbouring similar rabbit populations, and to eliminate the rabbits on each area by one of the two methods it is wished to compare. Unfortunately, it is not easy to find discrete populations of rabbits and, whereas for the brown rat we have a reasonably satisfactory method of measuring a population (Chitty, 1942), no method of census for the European rabbit yet exists. Under conditions such as occur in Great Britain, rabbits are not readily prebaited. Neither is the number of holes an indication of the number of rabbits dwelling in a warren, since holes may be dug by rabbits living 'rough' (i.e. above ground like hares); nor is the number of droppings a criterion of present rabbit abundance, since dung may persist almost unchanged in appearance for some weeks.

Although rabbits are trapped, ferreted, gassed, snared and shot in all parts of Great Britain, there is little information on the relative efficiency of the different methods of control, and only a handful of references to the subject (Sharpe, 1918;

Buckley, 1935; Read, 1939; Lockley, 1940; Middleton, 1940*a*; 1940*b* unpublished; Worden & Phillips, 1946–9 unpublished; Southern, 1948).

In March, 1949, a small experiment was done in Wales to compare cyanide gassing (using pump and spoon) with ferreting (Thompson, 1950). Approximately equal lengths of similarly infested hedge-banks, with little growth, were treated by each method and, under these conditions, gassing and ferreting were equally successful. The effectiveness of treatment in this experiment was judged by the re-opening of blocked holes during three subsequent weeks and, towards the end of this period, it was fairly certain that rabbits were immigrating from surrounding untreated hedges. Such re-invasion of relatively clear areas is a familiar complication in pest-control studies.

The fencing in of experimental areas with rabbit-proof netting is therefore desirable when making comparative tests and it was suggested, during discussions with the Forestry Commission, that experiments might be made in small forestry compartments, which are always fenced in and cleared of rabbits before planting. A site was eventually chosen in the King's Forest, Wordwell parish, Bury St Edmunds, West Suffolk. It consisted of 200 acres of grass-heath, fenced with rabbit-proof netting in the standard Forestry Commission manner* and divided by another fence of rabbit netting into two parts (A and B) of 80 and 120 acres respectively. There were two overgrown pits in the smaller area and a scattering of small pine trees at the north end. The larger area contained a very minute hill (Traveller's Hill—a tumulus) bearing a crown of adult Scots pines, a 12-acre wood (Traveller's Hill Plantation) containing principally Scots pine, two overgrown pits, a small chalk pit (not marked on the map) and a scattering of small pine trees in its south-east quarter. The soil throughout was sandy loam and contained numerous flints. Rabbit burrows were scattered over the whole area in small groups, the excavated, reddish soil making them clearly visible. There was a slightly greater concentration of burrows in the south-east pit in area A and the south-west pit in area B.

Description of experiment

(*a*) *General*

The present experiment was designed to compare the efficiency of gin trapping (in holes) in an area A with that of ferreting in an area B (Fig. 1). It was intended further to compare the efficiency of the two methods of applying cyanide, by pump gassing the residual rabbits of area A and spoon gassing those of area B. Both areas were treated concurrently, trapping usually occupying the mornings and ferreting the afternoons.

* Wire netting 1½ in. mesh, 30 in. high, with a further 12 in. turned outwards, flat on the ground, and held down by grass sods 12 in. square and 4 in. thick at intervals of about 12 in. The netting was supported by posts at 15 ft. intervals.

The experiment did not go entirely according to plan, owing to the re-opening of gassed holes by surface-dwelling (rough) rabbits on area A, and recourse had to be made to the use of 'dog and gun'. Before spoon gassing on area B, rough rabbits were coursed with dogs and shot, and subsequent gassing was successful. No comparison between the two gassing techniques could, however, be made, especially as it was found impracticable to dig out pump-gassed rabbits, owing to the depth of the burrows.

Fig. 1.

All rabbits recovered were sexed and most were weighed, dissected and weighed unpaunched. They were also examined for damage, coprophagy, parasites and, if females, for pregnancy and lactation.

(*b*) *Procedure in area A*

Two trappers used 144 no. 20 Dorset Wire gin traps between them. Traps were set in the holes and visited every morning, a varying proportion being moved on every day to fresh holes so as gradually to cover the whole area. All traps were taken up on Saturday morning and re-set on Monday. At the beginning of the experiment seventy-two unknotted wire snares were used for 7 days (because of poor trapping conditions) but they caught only nineteen rabbits and their use was discontinued.

TABLE 1. *Area A: rabbits recovered by various methods*

Method	♂	♀	Total	Man hours	Sex ratio ♀♀ (♂♂ = 100)	Rabbits per man hour
Gin trapping	106	106	212	210	100	1·1 [1·4]*
Snaring	9	10	19		111	
Pump gassing	2	1	3	53†	—	—
Dog and gun	14	8	22	17	57	1·3
Dog (alone)	—	—	—	4	—	—
By other means	2	1	3‡	—	—	—
Totals	133	126	259	284	—	—

* The figure in square brackets is obtained by making a correction for time wasted at week-ends lifting and relaying all traps and snares, i.e. half the time so spent (44 hr.) is subtracted from man hours.

† Includes 11 hr. spent digging out rabbits.

‡ Two killed by stoats and one caught by hand.

TABLE 2. *Area A: pump gassing*

		Number of holes gassed									
Date	Days of operation	1st time	2nd time	3rd time	4th time	5th time	6th time	7th time	8th time	9th time	10th time
17. x. 49	1	77	—	—	—	—	—	—	—	—	—
20. x. 49	4	—	33	—	—	—	—	—	—	—	—
21. x. 49	5	—	—	12	—	—	—	—	—	—	—
22. x. 49	6	—	—	—	19	—	—	—	—	—	—
24. x. 49	8	—	—	—	—	23	—	—	—	—	—
25. x. 49	9	—	—	—	—	—	9	—	—	—	—
26. x. 49	10	—	—	—	—	—	—	8	—	—	—
8. xi. 49	23	—	—	—	—	—	—	—	57	—	—
30. xi. 49	45	—	—	—	—	—	—	—	—	19	—
16. xii. 49	61	—	—	—	—	—	—	—	—	—	16

Between 26. x. 49 and 8. xi. 49 many holes were re-opened from the outside by surface living rabbits. Thirty-eight rabbits trapped and sixteen shot.

Between 8. xi. 49 and 30. xi. 49 twenty rabbits trapped and six shot.

Between 30. xi. 49 and 16. xii. 49 two rabbits trapped.

The experiment was begun on 4 October 1949 during a period of semi-drought and for the first 4 days few rabbits were caught, probably owing to the difficulty of adequately concealing the traps. This is commonly the case when the soil is dry and seeps down between the plate and jaws of the trap, partially exposing it. Rain fell on the night of 10/11 October, however, and there was a much heavier catch on that and the following night (see Fig. 2). A total of 231 of the 259 rabbits taken on the area between 4 October and 4 December was removed by trapping and snaring, and 155 of the 231 were caught by 15 October. Two rabbits were killed by stoats, *Mustela erminea*, and one picked up by hand; two stoats were caught in traps.

Since the traps had now been moved over the whole area, and holes blocked on moving, re-opened holes were then pump-gassed for the first time on 17 October (Table 2) and six more times during the next 9 days. During this time a small

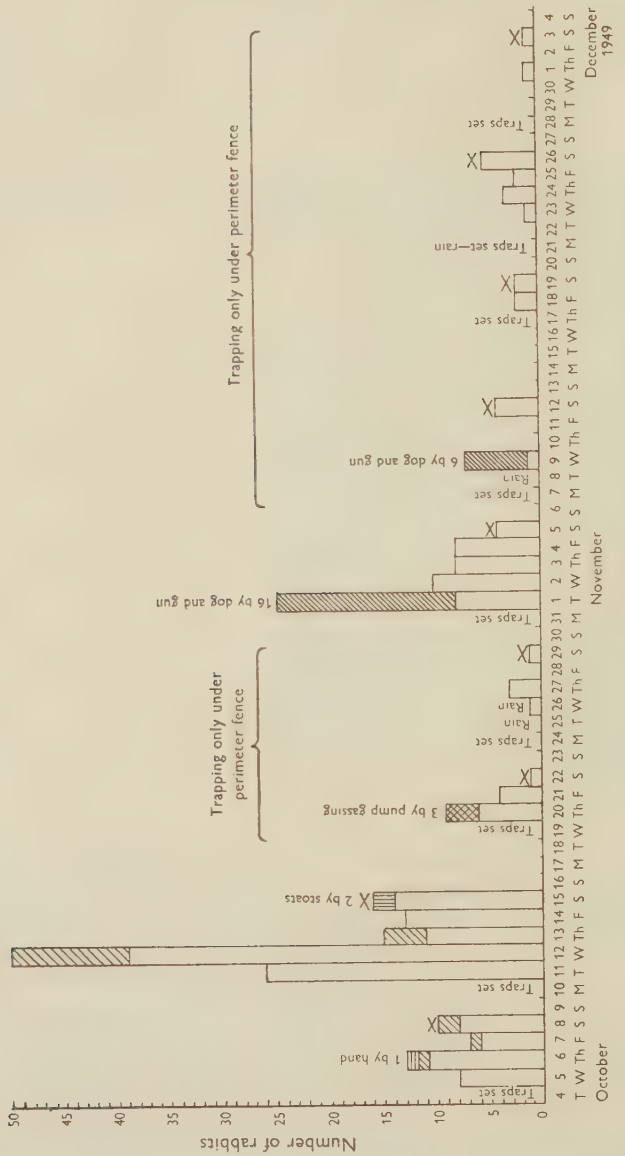


Fig. 2. Daily catch by all methods on Area A.

Key: gin trapping, ☐; dog and gun, ▨; pump gassing, ▩; traps lifted, ×; snared, ▨; stoat, ▥; by hand, ▤.

number of traps was set round the perimeter of area A and sixteen rabbits caught in holes made under the netting: some of these rabbits came from outside the experimental area and some from area B. It was proposed to dig out all the pump-gassed burrows and an attempt was made, but their depth was so great (over 9 ft. vertically down) that it was abandoned. Three gassed rabbits were recovered. At the seventh time of pump gassing there were still eight holes which had been re-opened (out of an original seventy-seven) and it was apparent that most of these were being opened from the outside by surface-living rabbits. On 1 November, the area was therefore hunted with a dog, and sixteen rabbits shot. Gin traps were also set in all re-opened holes and thirty-eight rabbits caught. Further pump gassing had no apparent effect and six more rabbits were shot on 9 November. Traps were set round the perimeter only for the next month (excluding weekends) and twenty rabbits caught. Area A remained almost rabbit-free during this period and was dogged on 19 November without starting any rabbits. Subsequently nineteen, and then sixteen holes were pump gassed. Most of these holes were probably made by visiting rabbits that had burrowed under the netting and, later, fallen victims to the perimeter traps. In area A, 24 lb. of the cyanogenetic product Cymag were used.

(*c*) *Procedure in area B*

Two men using four ferrets, three loose and muzzled and one leashed unmuzzled, caught rabbits fairly steadily in area B for 8 weeks from 6 October, working mainly in the afternoons (Fig. 3). Ferreting produced one rabbit per man hour, 354 being ferreted (Table 3). Very few rabbits bolted and it was necessary to do a great deal of digging.

After 8 weeks of part-time ferreting, about three-quarters of area B had been treated and it was decided to spoon-gas the residue. Profiting from the experience of area A, the 'rough' rabbits were first disposed of by hunting with dog and gun; because of the wood this was considered particularly necessary. Eighteen rabbits were shot on 2 December. All rabbit holes (2080) were then blocked with earth, and re-opened holes (848) were gassed in three main groups, each with two subgroups (Fig. 1; Tables 4 and 5). Re-gassing was carried out up to four times in some groups.

TABLE 3. *Area B: rabbits recovered by various methods*

Method	♂	♀	Total	Man hours	Sex ratio ♀♀ (♂♂ = 100)	Rabbits per man hour
Ferreting	128	226	354	360	177	1·0
Spoon gassing	32	21	53	140*	66	[0·5]†
Dog and gun	22	7	29	14	32	2·1
By other means	3	2	5‡	9	—	—
Total	185	256	441	523	—	—

* Includes 43 hr. spent digging out rabbits—subtracted when calculating last column.

† The figure in square brackets is given on the probable assumption that all rabbits killed by spoon gassing die close to the gassed holes.

‡ One killed by stoat and four by dog.

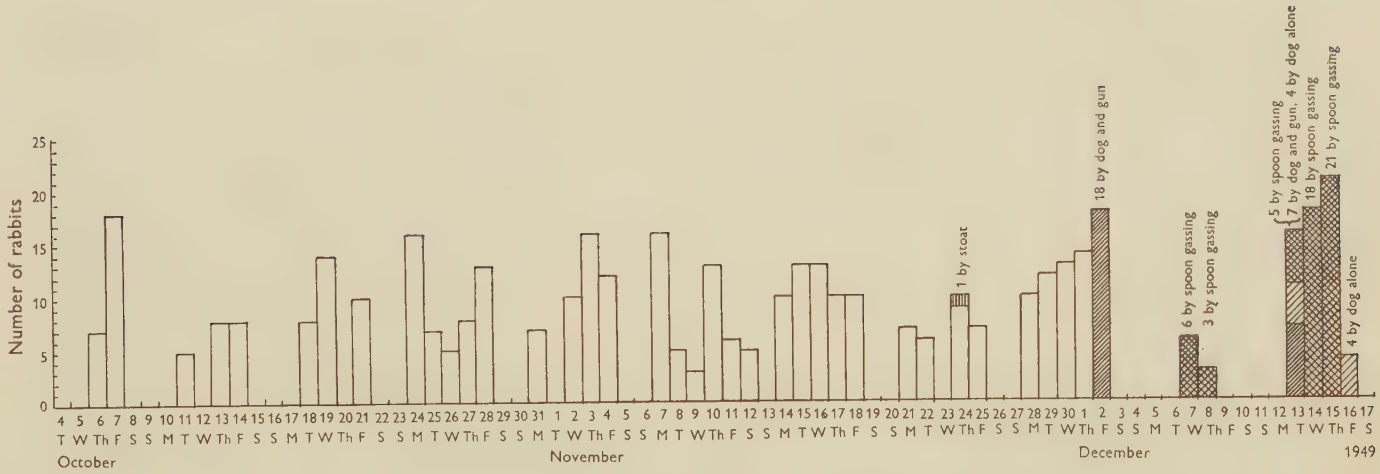


Fig. 3. Daily catch by all methods on Area B.

Key: ferreting, ☐; dog and gun, ▨; spoon gassing, ▩; dog alone, ▨; stoat, ▥.

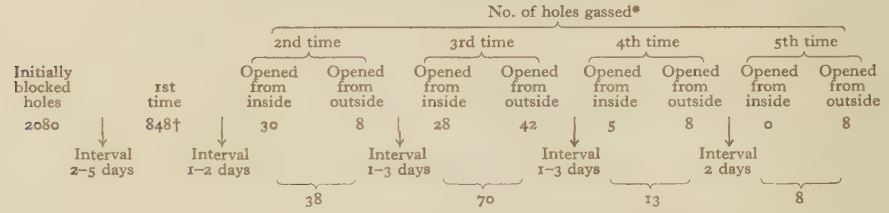
TABLE 4. *Area B: spoon gassing*

Group	Days of operation	No. of original holes blocked	Re-gassing: Re-opened holes initial gassing	Re-gassing: Holes re-opened: From inside	Re-gassing: Holes re-opened: From outside	Re-gassing: New holes: Opened from inside	Re-gassing: New holes: Opened from outside	Re-gassing: Overlooked holes	Rabbits recovered: ♂	Rabbits recovered: ♀	Rabbits recovered: Total
1	1*	1000	—	—	—	—	—	—	—	—	—
	3		144	—	—	—	—	—	—	—	—
	4		—	2	1	4	—	7	—	—	—
	5		—	—	1	1	—	1	4	2	6
			144	2	2	5	—	8	—	—	—
2	1		—	—	—	—	—	—	—	—	—
	4		73	—	—	—	—	—	—	—	—
	5		—	—	—	2	—	—	2	1	3
	6		—	—	—	—	—	—	—	—	—
			73	—	—	2	—	—	—	—	—
3	1	330	—	—	—	—	—	—	—	—	—
	6		131	—	—	—	—	—	—	—	—
	8		—	1	2	—	—	—	—	—	—
	11		—	5	10	—	—	—	—	—	—
	12		—	1	—	—	—	—	3	2	5
			131	7	12	—	—	—	—	—	—
4	1		—	—	—	—	—	—	—	—	—
	6		126	—	—	—	—	—	—	—	—
	8		—	5	2	—	—	4	—	—	—
	11		—	5	9	—	—	—	—	—	—
	13		—	2	3	—	—	—	5	3	8
			126	12	14	—	—	4	—	—	—
5	1	750	—	—	—	—	—	—	—	—	—
	4		145	—	—	—	—	—	—	—	—
	6		—	4	—	4	2	—	—	—	—
	8		—	6	11	—	—	—	—	—	—
	10		—	—	2	—	—	—	3	4	7
			145	10	13	4	2	—	—	—	—
6	1		—	—	—	—	—	—	—	—	—
	4		216	—	—	—	—	—	—	—	—
	5		—	1	—	7	1	—	—	—	—
	7		—	9	11	2	—	—	—	—	—
	9		—	—	—	—	—	—	1	2	3
	10		—	2	3	—	—	1	14	7	21
			216	12	14	9	1	1	—	—	—
Totals		2080	835	43	55	20	3	13	32	21	53

* Day on which original holes blocked.

N.B. Holes initially blocked 2–6 December. Final inspection 16 December 1949: found eight holes, re-opened from outside, four in wood and four in block 6; four rabbits caught by dog in block 6.

TABLE 5. *Area B: spoon gassing, incidence of hole re-opening*



* Includes thirteen holes overlooked at initial gassing, but subsequently gassed and not re-opened.
† After the initial gassing, the re-opened holes and new holes (bursts out) are treated together.

After the first gassing most re-opened holes were opened from the inside, whereas after the second gassing a larger number were re-opened from the outside (Table 5). This suggested that some rabbits which had escaped gassing were now living entirely above ground. Area B was therefore again coursed with a dog on 13 December and seven rabbits were shot, and four caught by the dog. After this, hole re-opening was negligible. Four more rough rabbits were caught by the dog on 16 December, the last day of the experiment.

All gassed holes were dug out, after a period of not less than 48 hr., and a total of fifty-three bodies recovered (Table 4); 42 lb. Cymag were used in area B, which was virtually rabbit free at the end of the experiment.

DISCUSSION

A glance at the histograms shows the very different course of events on the two areas. Whereas on A there was a high initial catch of rabbits in gin traps, ferreting on B yielded a slower and steady return, fluctuating with the time spent daily on the area. Neither area could have been completely cleared by trapping or ferreting alone, owing to the presence of surface-living rabbits.

A comparison of the returns in terms of rabbits per man hour is given in Tables 1 and 3. There was virtually no invasion of rabbits into area B under the netting (partly because a Forestry Commission trapper was working on the west boundary) and the figure of 1·0 rabbit per man hour for ferreting is a true one. The figure for gin trapping is more doubtful. Time spent on gin trapping and snaring includes 88 hr. (out of 210) spent merely lifting traps (and snares) on Saturdays and re-setting them on Mondays. It also includes all time spent patrolling the perimeter fences and trapping in burrows beneath them. There was a considerable amount of immigration into area A, and thirty-six rabbits were caught at the perimeter. The lowest possible figure for gin trapping is thus 1·1 rabbits per man hour. An allowance for time wasted at weekends* raises the figure to 1·4, and 155 rabbits, out of a total of 259 caught in area A, were trapped and snared during the first 9 days, yielding

* Most trappers manage to visit their traps on Sundays and so do not take them up at the weekend; the men on this job lived too far away to do this.

1·9 rabbits per man hour for that period. A fall in the returns from trapping as rabbits become scarcer is, of course, to be expected but a figure of about 1·5 rabbits per man hour is probably valid.

Middleton (1940*b*) records a catch of 900 rabbits in gin traps on an estate at Wasperton in Warwickshire by one trapper in 13 weeks. Working a 6 day week, this is an average of 11·5 rabbits a day, or about 1·5 per man hour.

It is not known how many rabbits were killed by pump gassing, but there is little doubt that this method was almost a complete failure, owing to the presence of surface-living rabbits, twenty-two of which were later shot on area A. This is not, of course, a condemnation of pump gassing as a method, but indicates its inappropriateness under these conditions.

Spoon gassing in area B was successful, largely because it was carefully integrated with the removal of rough rabbits, thirty-three of which were caught by dog and gun. The method was expensive in time, however, yielding only 0·5 rabbits per man hour. Although it is by no means the usual practice in this country, it is worth noting that rabbits are always dogged prior to fumigation in Australia (Ratcliffe, 1950).

In the 200 acres of the experiment, 700 rabbits in all were caught; the sexes were in the proportion of 100 males to 120 females. There were more rabbits absolutely and per acre on area B, but the difference was not great. There were almost equal numbers of males and females on area A (100:95) and equal numbers of each sex were caught by gin trapping (Table 1). There is no experimental evidence in support of the contention that buck rabbits are more frequently caught in gin traps than are does.

In area B there were fewer males than females (100 : 138), but the disparity between sexes caught by ferreting was greater than this (100 : 177; Table 3). Appreciably more males than females were dug out after spoon gassing (100 : 66).

It is significant that, in both areas, many more male than female rabbits were caught by dog and gun, the ratio being 100 : 57 in area A and 100 : 32 in area B. The figure for B is particularly interesting because of the excess of females over males on that area. These findings confirm the belief, held by many experienced rabbiters, that bucks are more inclined to live above ground and are mainly responsible for the re-opening of blocked holes from the outside.

Although it was not possible to make an initial count of the number of rabbit holes in the experimental areas, some information on the relation between numbers of holes and numbers of rabbits was obtained. In area A, surface-living rabbits so complicated pump gassing that the numbers of holes re-opened bore little relation to numbers of rabbits present.

In area B, ferreting with hole blocking afterwards proceeded over groups 3–5 (Fig. 1). Only a small part of the plantation was ferreted, so that the 1000 holes initially blocked before gassing (Table 4) had suffered little previous interference, although the wood had been hunted. Only 217 of these holes were re-opened at the time of the first gassing, 3–4 days after blocking. Nine gassed rabbits were recovered,

giving a ratio of 24 : 1 re-opened holes to rabbits. In groups 3 and 4 (which had been ferreted) 257 out of 330 holes were re-opened and thirteen gassed rabbits were recovered, giving a ratio of 20 : 1. In groups 5 and 6 (only group 5 had been ferreted) 361 out of 750 holes were re-opened and thirty-one gassed rabbits recovered, giving a ratio of 12 : 1. While the larger number of rabbits recovered from groups 5 and 6 may be attributed to group 6 not having been ferreted, this does not explain the lower ratio of holes re-opened to rabbits recovered. While it may have been due to rabbits in the unferreted area living in denser communities, owing perhaps to emigration from the ferreted areas, only a more careful study of individual warrens will solve problems such as this.

No young rabbits were caught. No female rabbits were found to be lactating and only one was pregnant, and its embryos were dead *in utero*.

Records of coprophagy showed that soft faeces were found predominantly in the stomachs of ferreted and shot rabbits, while they were almost completely absent from trapped and snared rabbits. The findings of Taylor (1940) and Southern (1942), that wild rabbits pseudo-ruminate during the day, in contrast to domestic rabbits which do so at night, are thus supported.

We are grateful to the Forestry Commission for making available and netting the experimental areas and for the generous help and advice of their staff.

The West Suffolk Agricultural Executive Committee gave every facility, including the services of their staff.

REFERENCES

BUCKLEY, W. H. (1935). Report on a solution of the rabbit problem: cyanide gassing. *Vet. J.* **91**, 210–15.

CHITTY, DENNIS (1942). A relative census method for brown rats (*Rattus norvegicus*). *Nature, Lond.*, **150**, 59.

LOCKLEY, R. M. (1940). Some experiments in rabbit control. *Nature, Lond.*, **145**, 767–9.

MIDDLETON, A. D. (1940*a*). *The control and extermination of wild rabbits*, pp. 1–28. Oxford, England: Bureau of Animal Population.

MIDDLETON, A. D. (1940*b*). Bureau of Animal Population. Report no. 2*c*, mimeographed.

RATCLIFFE, F. N. (1950). Personal communication.

[READ, E. C.] (1939). The control of rabbits. *J. Min. Agric.* **46**, 588–92.

SHARPE, R. (1918). The taking of wild rabbits. *J. Bd. of Agric.* **25**, 281–98 and 435–44.

SOUTHERN, H. N. (1942). Periodicity of refection in the wild rabbit. *Nature, Lond.*, **149**, 553–4.

SOUTHERN, H. N. (1948). The persistence of hydrogen cyanide in rabbit burrows. *Ann. appl. Biol.* **35**, 331–46.

TAYLOR, E. L. (1940). The demonstration of a peculiar kind of coprophagy normally practised by the rabbit. *Vet. Rec.* **52**, 259–62.

THOMPSON, HARRY V. (1950). *Mammalian pest control research report*, no. 27, Min. Agric. and Fish., mimeographed.

WORDEN, A. N. & PHILLIPS, W. (1946–9). Rabbit research in West Wales. (Unpublished.)

(*Received* 14 *July* 1950)

THE CONTROL OF BLOWFLIES INFESTING SLAUGHTER-HOUSES

I. FIELD OBSERVATIONS OF THE HABITS OF BLOWFLIES

By A. A. GREEN

Department of Scientific and Industrial Research, Pest Infestation Laboratory, Slough, Bucks

The habits of blowflies infesting slaughter-houses have been studied to determine the conditions under which control measures are most likely to be effective. Flies found in such numbers as to be considered pests of economic importance were: *Calliphora erythrocephala* (Meig.) and *C. vomitoria* (L.); *Lucilia caesar* (L.), *L. illustris* (Meig.) and *L. sericata* (Meig.); and *Phormia terrae-novae* (R.-D.). All were found breeding profusely on slaughter-house refuse.

The reactions of adult blowflies to weather and the working conditions at the slaughter-house are recorded; the three genera react in differing degrees to relatively minor changes in weather conditions, and redistribution of populations caused by these changes is so rapid as to make difficult the accurate assessment of degree of infestation. It was found that blowflies normally do not settle on the internal fabric of slaughter-house buildings but tend rather to congregate on nearby vegetation. The preferences were studied of adult flies for certain types of meat, for particular parts of carcasses, and for meat exposed to sunlight.

Conditions favouring rapid development of larvae were determined and the rates of breeding recorded for the three genera when in natural competition and when breeding independently. The larvae of *P. terrae-novae* were found to be predacious on other species and each other. *Lucilia sericata* was found to oviposit and breed successfully in vegetable matter.

Blowflies overwinter as fully grown larvae which pupate only a short while before emerging in the spring.

Various methods are discussed whereby the findings of this investigation into the life history and habits of blowflies can be utilized in the control of these pests.

Introduction

During the summer of 1947 the attention of the Pest Infestation Laboratory was drawn, both by Local Authority officers and by the Infestation Division, Ministry of Food (M.O.F.)*, to the problem of fly control in slaughter-houses. Infestations were very heavy during this summer and, as an emergency measure, M.O.F. undertook treatment of some heavily infested slaughter-houses with a DDT wettable powder. At the same time M.O.F. arranged facilities for the Laboratory: (1) to observe slaughter-houses during and after treatment, (2) to make a general survey of the problem, and (3) to carry out experimental treatments. It quickly became apparent that the treatment, which is normally effective in control of houseflies,

* The Infestation Division was subsequently transferred, without change of function, to the Ministry of Agriculture and Fisheries.

was killing very few blowflies and that a new technique would need to be related to the behaviour of the insects and to the peculiar needs of the slaughtering industry.

There are in England and Wales 448 licensed slaughter-houses, ranging from the large municipal type, with buildings extending over as much as 20 acres, to the small rural slaughter-house which may be no more than an outhouse to a butcher's shop; it is almost certain that all of these have fly problems to a greater or lesser extent.

The severity of the problem cannot be judged merely by the amount of meat which becomes 'fly-blown' and contaminated. The enormous numbers of blowflies which are bred on the premises cause considerable local nuisance, range over surrounding districts, and may spread disease by contaminating foodstuffs. Hall (1948) discusses different methods by which blowflies may transmit disease, and various workers have shown that the types of enteric infection spread by blowflies are very numerous; *Bacillus coli*, *B. dysenteriae* and *Entamoeba coli* are among the more important organisms isolated. The virus of poliomyelitis has been isolated from collections of flies, in which blowflies predominated, from infested areas by Paul, Trask, Bishop, Melnick & Casey (1941), Sabin & Ward (1941), Paul & Trask (1942), Trask & Paul (1943) and Trask, Paul & Melnick (1943). Melnick & Penner (1947) found that *Phormia regina* and *Lucilia sericata* were present in all positive batches of flies taken from epidemic areas and showed that the virus was present in the excreta of some blowflies 3 weeks after they had fed on infected material. Blowflies bred at slaughter-houses may well invade sheep-rearing districts and increase the sheep 'strike' problems; Gurney & Woodhill (1926) showed that blowflies can range up to 10 miles.

This paper describes studies to determine the circumstances under which control measures are most likely to be effective. In all, twenty-two slaughter-houses in twelve counties have been visited; some on one occasion only and one more than 100 times.

General conditions in slaughter-house premises

In basic design a slaughter-house consists of: (1) *the lairage*, where animals awaiting slaughter are kept; (2) *the slaughtering bay*, in which killing and the separation of edible and inedible materials takes place; and (3) *the hanging room*, where edible meat and offal hangs until the body-heat has diffused, and grading and inspection are completed. The smaller slaughter-houses may combine the slaughtering bay and hanging room into one room and the larger ones are usually multiples of the basic design but have also their own chilling rooms for final storage. Unfortunately, there are no typical conditions in that there is no uniformity of type or style of building or situation. The slaughtering which was previously carried on by local private traders in numerous small slaughter-houses is, under the existing bulk-buying system, concentrated into fewer premises, which are often inadequate for their present volume of work.

An efficient system of disposal of the inedible materials and refuse is essential if serious fly infestations are to be avoided. The inedible materials consist largely of:

(1) *Condemned meat and offal.* This is usually stored in an outbuilding but often is heaped in an open yard. The period of storage varies from not more than one day, as at most large slaughter-houses, to 3–4 days as at some small rural slaughter-houses.

(2) *Inedible gut.* This is stored in open metal drums, usually in the yards, and the storage period varies much as for the condemned meat and offal.

(3) *Blood.* As much blood as can be saved during slaughtering is stored in tanks or small metal drums while awaiting collection. It is usually only at the large, well-organised slaughter-houses that collection is sufficiently frequent to prevent breeding of blowflies in this blood. Spilled blood is either washed down the drains or swept up with the refuse.

(4) *Refuse.* This consists of dung from the gut of slaughtered beasts, sweepings from the yards and the floor of the slaughtering bays, pig hair, waste pieces of wool and hide and sometimes congealed blood removed from traps in the drainage system. It is frequently tipped into a pit or open yard and may remain there for periods varying from 2 hr. to 8 days. This mixture forms an ideal food for all the blowflies commonly found in slaughter-houses and, because of fermentation temperatures, encourages very rapid development.

The premises visited were diverse from the spacious modern type with refuse storage tanks or bays that are easily cleaned and concrete yards that can be efficiently hosed down, to the crowded old type in which refuse is tipped in loose heaps and the cobbled or gravel yard cannot be properly cleaned.

It is very noticeable that, at the more modern premises where it is not difficult to maintain a high standard of cleanliness, staff are usually conscious of the fly nuisance and willingly apply control measures. At the older places, which even with the utmost effort could not be made to look clean and hygienic, there is an air of general frustration and a resigned acceptance of heavy infestations of flies.

Composition of fly population

The following flies infest slaughter-houses in such numbers as to be considered pests of economic importance:

Calliphora erythrocephala (Meig.) and *C. vomitoria* (L.) (blue-bottle blowflies). Both species occur although very seldom in large numbers. The population consists of a mixture of the two species with *C. erythrocephala* the more common; the generic name alone will be used when describing this mixed population. During the summer, when the blowfly population at slaughter-houses is at its highest level, *Calliphora* seldom forms more than 10%. Instances have occurred of *Calliphora* following the scent of meat, entering grilles and flying against a strong stream of air along exhaust ducts and so reaching chilling rooms.

Lucilia caesar (L.), *L. illustris* (Meig.) and *L. sericata* (Meig.) (green-bottle blowflies). In general, the *Lucilia* population has been found to consist of about 95% *L. sericata* and 5% *L. caesar* and *L. illustris*. Mixed populations of this sort will be

referred to by the generic name only and the two species *L. illustris* and *L. caesar*, only the males of which can be separately identified, will be grouped as *L. caesar*-group. *Lucilia* is by far the commonest blowfly in and around slaughter-houses and, during the summer, represents more than 80% of the total population. Despite this, it is believed that it is responsible for a relatively small proportion of 'fly-blown' carcasses. *Lucilia*, occurring as it does in such vast numbers, causes by far the greatest nuisance and is, almost invariably, the cause of complaints by sanitary inspectors and others.

Phormia terrae-novae (R.-D.). This blowfly usually forms only a small proportion of the slaughter-house population but has been known to form heavy infestations, particularly in early summer.

Musca domestica L. (the housefly). Although seldom seen in the slaughtering, hanging or chilling rooms, and therefore not strictly a pest of the slaughter-house, the housefly is frequently found breeding in large numbers in the refuse and becomes a nuisance in the offices, canteens and nearby dwelling houses.

In addition to the above, twenty-eight other species of flies were collected.

The nature of the blowfly population changes considerably with the season. During the 3 years in which records have been kept, the first blowflies to emerge have been *Calliphora erythrocephala*; they appeared at the beginning of March in 1948, at the end of March in 1949 and in mid-January during the mild winter of 1950. Where *Phormia* have occurred they have been only a few days later than *Calliphora* and have sometimes quickly built up a predominant population. *Lucilia* have been first seen during mid-April each year but their numbers have increased so rapidly that they were predominant by June and remained so during the summer. By October each year the numbers of *Lucilia* were declining and the last few flies seen were *Calliphora*.

It has proved to be extremely difficult to obtain accurate data on overall population densities because of the rapid movement of flies in relation to weather and local working conditions. The combination of warm sunny weather and a day of heavy slaughtering, when meat, offal, hides, etc., are present in quantity, provides optimum conditions for heavy infestation. On the other hand, few flies are attracted to a clean, empty slaughter-house irrespective of weather conditions, and few are attracted on a sunless day irrespective of working conditions. Rapid redistribution of population occurs when these factors change: it has frequently been observed that, whereas during a cool cloudy period a slaughter-house was apparently uninfested, several thousand blowflies have appeared within 10 min. of the sky clearing and the sun shining. Similar difficulties have been experienced in obtaining data on the species distribution in the general population because of the differing habits of the species. *Phormia*, like *Calliphora*, seem to be rather more resistant to cold and less influenced by sunlight than *Lucilia*. Furthermore, resting *Lucilia* and *Calliphora* tend to congregate on nearby vegetation, whereas *Phormia* disperse over a larger area and rest in a greater variety of places, except that, when sunning themselves, they congregate

on outside walls. Counts were made of flies resting on walls, and caught by net among the vegetation and over the refuse, at a time when the overall population was estimated to consist of about 45% *Lucilia*, 45% *Phormia* and 10% *Calliphora*. Counts, summarized in Table 1, illustrate the need for a considerable knowledge of the habits of the flies when assessing the degree of infestation of premises.

TABLE 1. *The effect of weather conditions on the distribution of a mixed population of adult blowflies*

Weather conditions	Distribution of blowflies		
	On refuse	On nearby vegetation	On outside walls
Cloudy and cold	None	More than 80% *Lucilia*	None
Cloudy and cool	More than 80% *Phormia*	More than 80% *Lucilia*	None
Warm and sunny	Normal population	Normal population	More than 90% *Phormia*

BEHAVIOUR OF BLOWFLIES

Adults

Resting habits

Blowflies entering slaughter-house buildings are usually attracted by the scent of the meat and fly in through an open door or window, particularly if they can follow a shaft of sunlight, and, after visiting the meat, fly towards the light and so leave without settling on the fabric of the building. This accounts for the previously mentioned failure to control blowflies by treating the walls with DDT dispersible powder. Occasionally blowflies fly through a doorway to the meat and are trapped, when trying to leave, because of the light attraction of a skylight or window on the sunny side of the building. Under these conditions blowflies are found in large numbers, buzzing against the glass or wire mesh.

At night time and during cool, cloudy weather, very few *Lucilia* or *Calliphora* can be found in the yards of the slaughter-houses or in, or on, any part of the buildings but searches have shown that they tend to rest on nearby vegetation where they remain clustered even during continuous, heavy rain.

Although some *Phormia* rest on vegetation, they do not confine themselves to these places to the same extent as *Lucilia* and *Calliphora*; in cold, cloudy weather they were found resting singly in old tins, cracks in walls and between the boards of some old huts. *Phormia* also have a much greater tendency to rest on sunlit outside walls than have other blowflies infesting slaughter-houses. Table 2 illustrates this point with the results of four counts, each of 100 randomly selected flies which were resting on outside walls, made during a period when it was estimated that less than 50% of the local fly population was *Phormia*. Occasionally, during hot sunny weather, *Lucilia* and *Calliphora* have been observed resting in large numbers on outside walls which faced the sun and were immediately behind refuse, gut, etc.: they seem to do this only when gorged.

Table 2. *Counts of adult blowflies resting on sunlit, outside walls*

Date	*Phormia*	*Lucilia*	*Calliphora*
19. vii. 48	97	2	1
20. vii. 48	98	2	0
4. viii. 48	96	4	0
7. viii. 48	96	3	1

Reaction to sunlight

Sunlight has a very great influence on the behaviour of blowflies, and of *Lucilia* in particular. During sunny weather they fly freely, but during cool, cloudy weather they are seldom a nuisance. However, a prolonged period of fine weather is not necessary for the build-up of a heavy infestation of adults because of the redistribution of population which occurs within a few minutes of a change in weather conditions.

Blowflies have never been seen in large numbers on carcasses, except when the meat was hanging in direct sunlight but small numbers of females were seen to fly into dimly lit sheds and hanging rooms to oviposit on the meat. The need to oviposit seems to be the stimulus which temporarily reverses the normal reaction of the fly to sunlight, a conclusion reached by Graham-Smith (1916) and Wardle (1930).

Meat preferences

It has been found that blowflies are particularly attracted to some materials and will seek these in preference to all other types of meat.

(1) *Gut* (*edible and inedible*). This is probably the most attractive material to blowflies found in slaughter-houses. It attracts them both for feeding and oviposition and is liable to become heavily infested with eggs within only a few minutes of removal from the carcass.

(2) *Cut surfaces*. Blowflies show a marked preference for the cut surface of meat; *Calliphora* have been frequently observed ovipositing on such fresh meat but *Lucilia* appeared only to feed.

(3) *Kidney*. The kidney flap, particularly of sheep, is probably the most attractive part of the hung carcass; it is common practice in the meat trade first to examine this area under the kidney when looking for evidence of fly-blown meat. On almost all of the occasions during this investigation when *Lucilia* were known to have oviposited on a freshly slaughtered beast the eggs were deposited in the kidney region. *Calliphora* are also attracted to the kidneys but will oviposit on any cut surface.

(4) *Edible offal* (*hearts and livers*). During the early part of the investigation the opinion of various slaughter-house managers was found to differ on the attractiveness of offal, particularly liver, to blowflies; at some slaughter-houses it was reported that livers never became fly-blown, whereas at others it was said that this material presented the greatest of the infestation problems. It was found that, where this offal was put in refrigerating rooms within 3 hr. of slaughter, no infestation occurred

but, where this was not possible and the offal remained in the hanging rooms overnight, very heavy infestations of eggs resulted. Observation has shown that such edible offal does not become highly attractive to either feeding or ovipositing flies unless left in the hanging room more than 3 hr.

Larvae

Reaction to light

Patten's (1916) laboratory experiments showed that the photosensitivity of larvae of *C. erythrocephala* was negative throughout their life but was less so at an early age and at the migratory phase. Field observations have revealed that the larvae of all the species of blowflies breeding in slaughter-houses are negatively phototropic except for a short period during the first stage. For a few hours after hatching the larvae tend to remain and feed in the immediate vicinity of the egg batch, regardless of the light, but very soon migrate to darker areas and avoid the light for the remainder of the larval life.

Migration

Reports by the staff of slaughter-houses, and our own observations, indicated that migration of larvae seeking pupation grounds took place largely at night. Three experiments were made to obtain figures on the relative numbers of larvae migrating in the day and at night. In each experiment 25–30 lb. of refuse (dung, hair, blood, etc.) were exposed in a slaughter-house yard for 24 hr. and then brought in a bin back to the Laboratory. The infested material was tipped on to a metal sheet suspended above a larger metal tray, so as to trap migrating larvae which fell from the sheet. The refuse, which if left unchecked would be spread by the movement of the larvae, was contained by a fence of woven wire (6 meshes/in.) $2\frac{1}{2}$ in. high and 3 in. from the edge of the metal sheet; a second, similar fence round the edge of the sheet helped to prevent debris, carried through the inner fence, from falling over the edge and into the bottom tray. This arrangement meant that the risk of trapping larvae which had not intentionally left the food was largely removed because all trapped larvae had left the food by passing through a woven wire barrier, crossed an open space 3 in. wide, and passed through a second woven wire barrier before dropping into the tray below. The whole apparatus was contained in a small, metal-roofed hut having coarse stockinet walls which allowed free passage of air but prevented secondary infestation of the refuse.

Larvae were removed from the tray and counted at 8 a.m. and 4 p.m. G.M.T. each day; these times did not, of course, divide the day into light and dark periods but were most convenient for working arrangements. Separate observations before 8 a.m. and after 4 p.m. without making actual counts, showed that the larvae collected at 8 a.m. had almost all migrated during the hours of darkness. Recording thermographs gave records of the normal shade temperatures and the internal temperature of the refuse. Results of the three experiments are given in Table 3.

TABLE 3. *Results of three experiments on the tendency of blowfly larvae to migrate at night*

Age of infestation (days)	Period of day	Temperature of refuse (°F.)		Normal shade temperature (°F.)		No. of larvae migrating
		Max.	Min.	Max.	Min.	
4	Day	73	72	—	—	0
	Night	73	62	—	—	0
5	Day	82	70	—	—	50
	Night	92	59	—	—	5,050
6	Day	93	68	—	—	40
	Night	83	53	—	—	6,545
7	Day	82	60	—	—	0
	Night	68	54	—	—	12
3	Day	103	72	93	74	0
	Night	98	75	86	73	162
4	Day	87	76	95	75	45
	Night	82	55	86	64	3,325
5	Day	76	58	86	66	102
	Night	70	51	79	61	480
6	Day	73	56	82	66	10
	Night	67	49	77	59	0
2	Day	92	65	77	62	0
	Night	94	92	73	60	0
3	Day	104	94	83	65	265
	Night	102	89	75	66	5,550
4	Day	100	89	80	66	2,775
	Night	89	66	71	61	6,660
5	Day	69	66	66	63	56
	Night	69	61	66	59	0
					Total Day	3,343
					Night	27,784

TABLE 4. *Results of two experiments on the migration by night of blowfly larvae kept entirely in darkness*

Age of infestation (days)	No. of larvae migrating	
	Night	Day
3	791	134
4	758	68
5	1,033	15
6	46	0
4	3,428	1,513
5	29,100	82
6	1,825	231
7	2,465	213
8	825	121
Total	40,271	2,377

It is clear that the main migration of larvae took place at night. Records of temperature show that the periods of mass migration were not related to extremes of temperature either inside or outside the food.

Two similar experiments were then made in a room kept in complete darkness to determine whether the migration of larvae at night was largely a measure of their reaction to light; 45 lb. of infested refuse was used in each experiment. In the second test the temperature was maintained at 25–28° C. so that migration could not be influenced by variable air temperatures. Results are given in Table 4.

It appears from these experiments that some factor other than light prompts the larvae to migrate during the night; it has not yet been possible to investigate the phenomenon further.

Larvae which have migrated from the food and have burrowed into the ground, preparatory to pupation, will remain there only if conditions are favourable. If the soil becomes waterlogged the larvae will return to the surface and migrate farther in search of more suitable pupation sites; in these circumstances there is a general tendency for them to travel uphill. The effect of a heavy rainstorm in the vicinity of a severe infestation can be quite startling; although not seen by the writer, he was told by reliable witnesses of the surface of the ground, at a railway siding handling slaughter-house refuse, becoming completely covered by tremendous numbers of larvae which migrated into houses and, being able to travel in a film of water on vertical surfaces, entered a signal box and even travelled almost to the top of telegraph poles and railway signals.

Breeding on slaughter-house premises

General

Blowflies have been found breeding on slaughter-house premises under a variety of conditions ranging from the infestation by vast numbers of larvae of some 70 tons of refuse, to small infestations by a few larvae of blood in cracks in concrete.

The following have been found breeding on slaughter-house premises: *C. erythrocephala* (Meig.), *C. vomitoria* (L.), *Lucilia caesar*-group, *L. sericata* (Meig.), and *Phormia terrae-novae* (R.-D.): all breed freely in slaughter-house refuse and inedible offal. All the observed infestations of tanks of blood have been by *Calliphora vomitoria*, the larvae floating on, or near, the surface while feeding and migrating by climbing over the side of the tank.

It has been generally considered that adult blowflies would oviposit only on, or near, materials having a high content of animal matter which the larvae needed for satisfactory development, although Thomsen & Hammer (1936) recorded *Lucilia sericata* breeding on pig-dung. Potgieter (1945) says that blowfly larvae can feed only on dead bait or sheep, and Cragg (1950) is inclined to the view that *L. sericata* cannot breed in dung. In July 1948, however, large numbers of larvae of *L. sericata* were found in fermenting pig-food which, so far as could be ascertained, consisted

only of vegetable matter; a sample of the infested material was brought back to the Laboratory for observation on larval development. In August 1948, *L. sericata* were observed ovipositing on fresh tripe-dung immediately after its removal from the slaughter bays; some of the eggs were collected and the larvae were reared on the wet mixture of grassmeal, bran, malt and yeast, which is used at the Laboratory for breeding houseflies. In both instances the larvae developed satisfactorily and gave rise to adults which appeared normal and laid viable eggs.

Oviposition

The adult female blowfly begins oviposition about 3–7 days after emergence; at 24° C. the comparative periods from emergence to first oviposition for the three genera are: *Calliphora*, 4–5 days; *Lucilia*, 4–5 days; *Phormia*, 6–7 days.

Oviposition occurs to the greatest extent during the hours of daylight but the habits of *Lucilia* and *Calliphora* seem to differ. Field observations at night were limited to three periods, each of only 1–2 hr., and were made on a population estimated to consist of 95% *Lucilia* and 5% *Calliphora*. They indicated that *Calliphora* quite commonly flew and oviposited during the night, spreading their eggs in ones, twos and threes over livers and the cut surface of carcasses, but that *Lucilia* seldom, if ever, did so. Under laboratory conditions it has been found that *Calliphora erythrocephala*, *Lucilia sericata* and *Phormia terrae-novae* will all oviposit in total darkness, although Wardle (1930) asserts that blowflies do not oviposit in the complete absence of light.

The commonly occurring species normally deposit their eggs in batches of 10 to 400 in crevices in the chosen larval food and, if possible, they will burrow deeply into the material; ovipositing females have been found 8 in. below the surface of loose refuse. If the material dries rapidly, or is moved so that the eggs are exposed to the drying action of sun and wind, a high proportion of the eggs fail to hatch. Davies & Hobson (1935) and Davies (1948) have shown that a high humidity is essential for the hatching of eggs of *Lucilia sericata*.

The egg period varies according to the age of the egg when laid and the temperature and humidity; most commonly, eggs hatch in 12–16 hr. If suitable material on which to oviposit is not available, the gravid female retains the eggs for as long as possible. Eggs laid under these conditions may hatch in a very short time; both *Calliphora erythrocephala* and *C. vomitoria* have been observed to deposit living, newly hatched larvae.

Larval development

Slaughter-house refuse will support very dense populations of larvae, particularly if the material is spread in a shallow layer so that heat can diffuse; under such circumstances 1 cwt. of refuse may support successfully 180,000–200,000 larvae. It has been found by both observation and experiment, that under favourable

conditions of food and temperature the larvae of *Lucilia* and *Phormia* can become fully grown and migrate within 3 days of oviposition.

When the larvae of *Calliphora*, *Lucilia* and *Phormia* were in natural competition, *Phormia* were by far the most successful; several instances occurred where, from material known to be heavily infested with larvae of *Lucilia* and *Phormia* in approximately equal numbers, the population surviving was about 5% *Lucilia* and 95% *Phormia*. Examinations of such cultures revealed the existence of many deflated larval skins and led to the conclusion that *Phormia* larvae are predacious and, in one instance, one larva was observed feeding on another. Several workers have demonstrated the inability of *Lucilia* to compete with other genera: Fuller (1934) showed 'that the larvae of *L. sericata* readily succumb to competition with other larvae'. Holdaway (1930) concluded that 'the greatest factor in the reduction of the fly population within the carrion is Dipterous competition and its associated predatorism'.

When fully grown the larvae usually migrate from the food to the pupation site: this is normally within about 20 ft. but under unfavourable conditions of very hard ground, larvae have been known to travel 80–100 ft.

Pupation and emergence

It has been found that blowfly larvae will pupate in almost any situation that is dark, and cooler and drier than the breeding medium but, if the ground is sufficiently soft and not waterlogged, the majority will burrow into the earth and pupate at 0–2 in. depth and, exceptionally, as deep as 6 in. Where soft earth is not available larvae will pupate in cracks in walls, under sacks, pieces of wood and various other objects.

If the food is relatively dry and not exposed to bright light, *Phormia* larvae do not always leave it but may pupate on the surface in a very typical manner.

At favourable temperatures the pupation period may be as little as 4 days. After emergence the three genera develop to the stage of flight at a very similar rate to that described by Fraenkel (1935) for *Calliphora erythrocephala* studied under laboratory conditions.

Rates of development

(1) *In natural competition*

Experiments were carried out in which all emerging flies were trapped and removed daily from refuse which had previously been exposed for 3 days in a slaughter-house yard. By this method the relative rates of development of the three genera of blowflies commonly occurring in slaughter-houses were determined by comparing the minimum times, from the setting-up of the heaps of refuse to the emergence of the first flies. It is possible that the actual periods for the life cycles were, in some instances, less than the figure given, because eggs were not laid until the second day of exposure but, because of uncertainty, it was necessary to date infestations from the time the refuse was deposited in the yard.

The figures given in Table 5 establish a definite order of development among the flies breeding in natural competition in the refuse.

TABLE 5. *The rates of development of* Phormia, Lucilia *and* Calliphora *when breeding in natural competition*

	Period (in days), for the development of the first adult flies		
Exp. no.	*Phormia*	*Lucilia*	*Calliphora*
12	9	13	19
13	16	19	22
14	16	17	21

The variation between the three experiments in the rates of development are attributable to weather conditions: in Exp. 12 the weather was hot and temperatures were over 80° F. for 8–10 hr. each day; whereas in Exps. 13 and 14, corresponding temperatures were seldom higher than 65° F. Results show that the order of emergence of blowflies when breeding in natural competition in slaughter-house refuse is: *Phormia*, *Lucilia* and *Calliphora*. Rates of development are similar to those quoted by Morrison (1948) but more rapid than those given by Wardle (1927), and would certainly result in more than the four generations per year which the latter estimated could breed in this country.

(2) *Without competition*

In order to obtain accurate data on the relative rates of development of *Calliphora erythrocephala*, *Phormia terrae-novae* and *Lucilia sericata*, the three species were bred independently but under similar conditions in a room maintained at 24° C. and 65% R.H. Ox liver was exposed for 6 hr. in the oviposition cages and was then kept for 24 hr. in closely covered dishes to maintain a high humidity and ensure a good hatch of eggs. The infested liver was then transferred to glass jars 7 in. high and 6 in. diameter and, as the larvae developed, more liver was added to ensure an excess of food at all times and so avoid any possibility of enforced early migration or pupation. When migration from the food began, a little peat was added for the

TABLE 6. *The rates of development of* Phormia terrae-novae, Lucilia sericata *and* Calliphora erythrocephala, *breeding at* 24° C. *without competition*

			Rate of development (in days) from time of oviposition					
Oviposition date	Blowfly species	Hatching period (hr.)	First migration	First pupation	Main pupation	First emergence	Main emergence	Life cycle, egg to adult
17. iv. 50	*Phormia terrae-novae*	20–25	7½	8½	9½	15	16	15–16
	Lucilia sericata	11–14	4½	9	15	16½	22½	16½–22½
	Calliphora erythrocephala	3–7	4½	8	13½	18½	21½	18½–21½
20. iv. 50	*Phormia terrae-novae*	19–25	7	8	9	13	16	13–16
	Lucilia sericata	10–14	5½	10½	—	19	24½	19–24½
	Calliphora erythrocephala	3–8	5½	8	14½	21	26	21–26

larvae to pupate in. Two experiments were made and the records of rates of development are summarized in Table 6.

When breeding on liver in separate cultures, the order of emergence of the three genera is similar to that obtained when breeding in natural competition in slaughter-house refuse, viz. *Phormia*, *Lucilia* and *Calliphora*. The rate of development is rather slower than for the flies bred in slaughter-house refuse, probably because the temperatures of small pieces of liver remained very much lower than those reached by fermenting refuse. It will be seen in Table 7 that *Phormia* eggs are relatively slow to hatch and the larvae spend a long time in the feeding stage, but as soon as feeding is completed metamorphosis to the newly emerged adult fly is fairly rapid. On the other hand, *Lucilia* and *Calliphora* eggs hatch quickly and larval feeding is of relatively short duration, but a long and variable non-feeding phase occurs before pupation which, together with a slightly longer pupal period, delays the eventual appearance of the adult.

TABLE 7. *Duration of four stages of development of* Phormia terrae-novae, Lucilia sericata *and* Calliphora erythrocephala, *when breeding at* 24° C.

	Approximate periods			
Blowfly species	Hatching (hr.)	Larval feeding (days)	Larval post-feeding (days)	Pupation (days)
Phormia terrae-novae	19–25	6–6½	1	5–7
Lucilia sericata	10–14	4–5	4½–9	7–9
Calliphora erythrocephala	3–8	4–5	2½–7½	8–13

OVERWINTERING OF BLOWFLIES

In the London area and Home Counties overwintering of blowflies has been studied to determine the method by which reinfestation of slaughter-houses occurs in the spring. All the information obtained showed that blowflies spend the winter as fully grown larvae which pupate only a short while before emergence. There was no evidence to indicate that blowflies can survive the winter as adults or puparia although Graham-Smith (1916) concluded 'that the very great majority of individuals pass the winter as pupae, or more rarely as larvae' and that some adults which emerge late in the autumn might survive until the spring.

Field observations

Calliphora

Adults of *C. erythrocephala* and *C. vomitoria* are active for a greater part of the year than the other commonly occurring species of blowflies, and the period of larval hibernation is relatively short. Oviposition continues until late autumn and feeding larvae have been found as late as mid-November although the majority of larvae have, by this time, migrated to their overwintering quarters. Hibernation takes place in the

ground, usually within an inch of the surface; under light rubbish, such as sacking or straw; or in cracks in walls, concrete, etc. The period of hibernation is usually 5–6 months, emergence normally beginning in early March but, as recorded by Graham-Smith (1916), small numbers emerge throughout the winter. During the mild winter of 1949–50 emerging adults of *C. erythrocephala* were found as early as mid-January, and several instances were recorded of their emerging when the air temperature was 0–2° C. Wardle (1927) found that *C. erythrocephala* emerge in March and April, as soon as the mean minimum ground temperature exceeds 5° C.

Lucilia

During the first two winters of the investigation observations gave no information on the overwintering habits of *Lucilia*, although Davies (1929), Holdaway & Evans (1930) and Hagmann & Barber (1948) had all shown that *L. sericata* could overwinter as fully grown larvae in the soil. From the large numbers of hibernating larvae, and samples of soil containing larvae, which were brought back to the Laboratory, the only adults to emerge were *Calliphora* and a small number of *Phormia*. In February 1950, samples of soil were collected from localities where *Lucilia* infestations were known to have occurred during the autumn of 1949. Some of these samples were kept at 20° C. to speed pupation and emergence and some were stored outdoors. Of the larvae kept at 20° C. some pupated within a few days and emerged as *Calliphora erythrocephala* but the remainder died. Dr E. A. Parkin examined the dead larvae and identified them as *Lucilia* sp.; further evidence was provided in the following May when several *Lucilia sericata* emerged from the samples stored outdoors. It has now been found that any attempt to shorten the hibernation of the larva of *L. sericata* normally results in its death, although Cousin (1929), after studying the factors governing the diapause of *L. sericata*, concludes that development can generally be resumed if a return is made to the external conditions existing before diapause. Hagmann & Barber (1948) found that fully grown larvae brought indoors in November failed to pupate, whereas those left in the soil and brought in during March pupated and emerged normally.

Observations show that *Lucilia* usually cease breeding in early October and that during this month the fully grown larvae burrow into the ground for hibernation: these larvae seem to burrow more deeply than *Calliphora* and have, so far, not been found under surface refuse or in cracks in walls, etc. The period of hibernation is about 6–7 months, there being little emergence before the following May.

Phormia

No heavy autumn infestations of *Phormia* have yet been met, and little information has therefore been obtained on their overwintering habits. In March 1948, however, a few prepupal larvae and some emerging adults of *P. terrae-novae* were dug from the ground near a slaughter-house midden. It seems that *Phormia* also overwinter as larvae in the soil.

Experimental work

Larvae

To study the behaviour of overwintering blowfly larvae and to determine more exactly the time of emergence of adults, about ½ cwt. of infested slaughter-house refuse was transported to the Laboratory during October 1949, and heaped in the centre of a level, recently dug patch of ground. The larvae were able to migrate from the refuse and burrow into the soil but, to discourage them from wandering too far, asbestos roofing slates were pushed into the ground so as to form a barrier about 6 in. high and enclose an area of 36 sq.ft. The experimental area was covered with a wire-netting frame as a protection against birds. Restricting the distance of migration did not impose unnatural conditions because larvae which climbed over the barrier burrowed into nearby soil and behaved in the same way as those in the experimental area.

As shown by Davies (1929) the larvae remained capable of movement during the winter and tended to burrow more deeply into the ground during frosty weather and to rise nearer to the surface when the ground became waterlogged. During the warm weather in March it was found that most of the larvae were in the top ½ in. of soil and that some were moving freely so near to the surface as to make surface markings similar to mole 'runs'. The *Calliphora* almost all pupated during the first

TABLE 8. *Blowflies trapped when emerging after overwintering as larvae in the soil*

	No. of blowflies emerging	
Date	*Calliphora*	*Lucilia*
3 April	2	0
5	6	0
6	10	0
8	18	0
14	9	0
17	10	0
20	62	0
22	1	0
24	5	0
29	1	0
2 May	1	1
4	0	1
9	0	3
10	0	4
11	0	2
13	0	3
16	0	15
18	0	13
19	0	3
22	0	16
30	0	9
2 June	0	2
Total	125	72

2 weeks in March but the *Lucilia* did not begin to pupate until the third week in March and continued until mid-April.

On 21 February 1950, small cages were fixed in the soil to trap some of the emerging adults. Results, which are summarized in Table 8, show that blowflies can overwinter in the soil as larvae which pupate and emerge in the spring. *Calliphora* emerge about 1 month earlier than *Lucilia* as shown by Wardle (1927). It was also found that no blowflies overwintered in the soil beneath the refuse or in the refuse itself; Hagmann & Barber (1948) obtained similar results when an infested carcass was left outdoors during the winter.

Adults

When, by November 1949, no information had been obtained on the overwintering habits of *Lucilia*, the possibility was considered that, although no adults had been found during winter, a small number were able to survive and form the nucleus of the next season's infestations. Five hundred adults of *L. sericata* were released, therefore, into a 7 ft. cube shed and left for the winter. The shed had walls of 1 in. thick timber covered with roofing felt, a corrugated iron roof and an earth floor and was made fly-proof by filling all cracks with cotton-wool; there was a door and a window. In order to provide shelter for resting flies some sacks were hung against the walls and a heap of straw placed in one corner of the floor. The flies, which had been bred under laboratory conditions from infested slaughter-house refuse collected in October, were released into the shed on 24 November 1949. In order to avoid a sudden change in temperature, the shed was heated to about 20° C. for the first 2 days and allowed to fall to normal temperature over the next 2 days; for the remainder of the winter it was unheated. On 4 January 1950, the shed was opened and only twelve flies found alive and on 29 March a further examination showed that none had survived; there were many dead flies on the floor. The minimum grass temperatures immediately outside the shed fell below freezing point fifteen times between 24 November 1949 and 4 January 1950, the lowest record being 19° F., and fifty times between 4 January 1950 and 29 March 1950, the lowest record being 12° F.

Although not conclusive the experiment showed that it is unlikely that *L. sericata* can overwinter in the adult stage.

Habits in relation to control

Impressions formed at the beginning of the investigation, that a special technique for control would need to be evolved which was related to the habits of the blowflies and to the peculiar needs of the slaughtering industry, have been confirmed. The investigation has indicated several methods by which knowledge acquired can be utilized and various possibilities are discussed below.

Control of adult blowflies

The adult blowfly, after entering buildings, normally settles only on the food thus precluding control inside the building: residual film application to the walls would be ineffective and space sprays would taint the meat.

Outside the buildings, chemical control could be effective only where flies congregate in large numbers as when feeding and ovipositing on waste materials, when resting, or when emerging from the soil after pupation. Baker & Schwartz (1947) have shown the importance of treating night resting places as well as daytime feeding places.

When feeding and ovipositing on waste material. Mixing the waste material with a poison or coating it heavily with a toxic film cannot be recommended, because almost all the inedible offal and some of the refuse is later processed as animal feeding stuff. Direct sprays, dusts or smokes, applied so as to drift over the material, might control flies which have settled on it or are flying near and at the same time lay down a deposit sufficient to affect or repel flies without risk of taint. Such measures, however, can give only temporary relief, particularly where fresh refuse is being added frequently.

When resting. Adult blowflies are probably best controlled while resting, because the sites can be treated without risk to edible materials and are more permanent than the refuse heaps. At night and during cool, cloudy weather, blowflies cluster on nearby vegetation which could be treated with a non-repellent insecticidal film. Outside walls, on which adults sun themselves, can also be treated with residual films.

When emerging. A high proportion of adult flies might be killed on emergence from the puparia by coating the soil in the vicinity of breeding grounds with a persistent insecticide: until their wings were functional, the emerging flies would have to walk on the treated surface.

Control of larvae

Control of blowflies in the larval stage is likely to prove difficult for the larva is not easily accessible during most of its life and is known to be relatively resistant to available contact insecticides.

When feeding. During most of the feeding stage the larva lives in a mass of soggy, wet food into which an insecticide cannot be efficiently mixed. Moreover, the insecticide must not reduce the value of the material for eventual use as a fertilizer of for animal feed.

Many observations of the death of large numbers of larvae buried more than 12 in. below the surface of refuse and thus exposed to high fermentation temperatures and accumulation of waste gases suggest that a practicable means of control would be the storage of refuse in deep pits or bins so constructed that fresh material would be tipped on top and the old collected from the bottom.

When migrating. Control of larvae during migration presents fewer problems because the larvae are out of their foodstuff and accessible to chemical control by treatment of the ground around infested material with a larvicide possessing residual toxicity. The use of direct sprays is scarcely practical as repeated applications would have to be given at night when the larvae were migrating.

Probably the most economical method of control would be trapping the larvae in trenches containing a larvicide but both yards and trenches would need to be suitably constructed from concrete, etc. The provision of attractive pupation sites, e.g. sacks or sawdust, which could be burned before adults emerged could also contribute towards control.

After migration. Larvae in the ground may be killed by heavy application of a cheap, toxic substance. Those which have not reached the prepupal state may, before treatment, be induced to return to the surface by soaking the ground with water. However, it is considered more practical to aim at control of the emerging adult.

Prevention of breeding

The most important approach to blowfly control in slaughter-houses is prevention of breeding on the premises and this can be done almost entirely by strict attention to hygiene, the general standard of which could be greatly improved by particular attention to the following:

(1) *The midden.* Blowfly eggs can develop to migrating larvae within 3 days, refuse should, therefore, remain on the premises for no more than this and, where possible should be cleared daily. Rotational storage and collection are recommended for handling large quantities of refuse and were adopted experimentally, during 1948 and 1949, at one very large slaughter-house which had previously suffered extremely heavy infestations of larvae. The midden yard was divided by brick walls into three bays which were filled with refuse as follows: bay 1, Tuesday and Wednesday; bay 2, Thursday and Friday; bay 3, Saturday and Monday. They were cleared completely in the same order and hosed down immediately after clearance. Refuse was thus normally removed when less than 2 days old and only a half-day's refuse remained in the yard over a week-end. During the two summers, only three larval infestations occurred and these were directly attributable to breakdowns in the collecting arrangements.

Such measures do not eliminate infestation but tend to prevent the build-up of an endemic population by transporting infested material to other districts. Nevertheless, by reducing the local adult fly population and by removing refuse before it decomposes to the state of maximum attraction to ovipositing blowflies, the refuse does not become heavily infested. Furthermore, a high proportion of the young larvae are almost certainly killed by the conditions of storage in depth during and after transport.

Any surface treatment of the refuse which, while not reducing its value for other purposes, would reduce or prevent oviposition would be of very great value.

(2) *Blood, inedible meat and offal.* These materials should be stored in outbuildings. The inedible offal should be kept in heavy, galvanized-iron bins, having strong lids with deep, overhanging rims, and not in the less robust type commonly supplied or in the open oil-drums sometimes used. All bins should be emptied every day or, at the latest, every second day and should be hosed out with water before re-use.

(3) *Destructible waste.* In addition to control of major breeding grounds, very strict attention should be paid to the daily burning of destructible waste, such as discarded aprons and other clothing, sacking and rags used for swabbing, blood-soaked sawdust, etc.; these can otherwise provide a breeding ground for large numbers of larvae.

Prevention of contamination

Edible meat and offal should be hung in shaded rooms, preferably fitted for high speed, forced ventilation. There is normally always sufficient shade to serve the needs of ovipositing blowflies, so that deliberate shading need not be expected to increase the risk of contamination with eggs. If possible, edible offal, especially livers, should be put in a chilling room within 3 hr. of slaughter, i.e. before it becomes highly attractive to ovipositing flies. All inedible materials attractive to adult blowflies should be stored as far as possible from the hanging rooms, so that flies will tend to be attracted away from the edible materials.

No one method, or group of methods, for control of blowflies in slaughter-houses will be applicable to the slaughtering industry as a whole but if detailed knowledge of the habits of the pests is coupled with a consideration of local conditions, the chances of successful control will be very considerably improved.

The author acknowledges the helpful advice given by Dr E. A. Parkin during the investigation and the preparation of this paper. Acknowledged also is the co-operation, freely given, of the various authorities concerned and particularly of Mr F. R. Cann of Infestation Division, Ministry of Agriculture and Fisheries; Mr E. F. McCleary of the Corporation of London, and Mr C. A. Harris of Meat and Livestock Division, Ministry of Food. Thanks are also due to Dr F. van Emden who identified many of the adult blowflies. Much help in the laboratory experiments was given by Miss Joyce Kane.

This work has been carried out as part of the programme of research of the Pest Infestation Laboratory, and this account is published by permission of the Department of Scientific and Industrial Research.

REFERENCES

Baker, W. C. & Schwartz, L. G. (1947). Preliminary studies on the control of blowflies with DDT. *Publ. Hlth Rep., Wash.*, **62**, 800.

Cousin, G. (1929). Les facteurs externes qui déterminent la reprise de l'évolution larvaire de *Lucilia sericata* Meig. et leur relation avec les influences extérieures qui ont provoqué la diapause. *C.R. Soc. Biol., Paris*, 101, **25**, 1117.

Cragg, J. B. (1950). Studies on *Lucilia* species (Diptera) under Danish conditions. *Ann. appl. Biol.* **37**, 66.

Davies, L. (1948). Observations on the development of *Lucilia sericata* (Mg.) eggs in sheep fleeces. *J. exp. Biol.* **25**, 86.

Davies, W. M. (1929). Hibernation of *Lucilia sericata* (Mg.). *Nature, Lond.*, **123**, 759.

Davies, W. M. & Hobson, R. P. (1935). Sheep blowfly investigations. 1. The relationship of humidity to blowfly attack. *Ann. appl. Biol.* **22**, 279.

Fraenkel, G. (1935). Observations and experiments on the blowfly (*Calliphora erythrocephala*) during the first day after emergence. *Proc. zool. Soc. Lond.* 893.

Fuller, M. E. (1934). The insect inhabitants of carrion: a study in animal ecology. *Bull. Coun. sci. industr. Res. Aust.* no. 82.

Graham-Smith, G. S. (1916). Observations on the habits and parasites of common flies. *Parasitology*, **8**, no. 4, 440.

Gurney, W. B. & Woodhill, A. R. (1926). Investigations on sheep blowflies. 1. Range of flight and longevity. *Sci. Bull. Dep. Agric., N.S.W.*, no. 27, 1.

Hagmann, L. E. & Barber, G. W. (1948). Overwintering habits of *Phaenicia sericata* (Mg.). *J. econ. Ent.* **41**, 510.

Hall, D. G. (1948). *The blowflies of North America.* Thomas Say Foundation (United States).

Holdaway, F. G. (1930). Field populations and natural control of *Lucilia sericata*. *Nature, Lond.*, **126**, 648.

Holdaway, F. G. & Evans, A. C. (1930). Parasitism a stimulus to pupation: *Alysia manducator* in relation to the host *Lucilia sericata*. *Nature, Lond.*, **125**, 598.

Melnick, J. L. & Penner, L. R. (1947). Experimental infection of flies with human poliomyelitis virus. *Proc. Soc. exp. Biol., N.Y.*, **65**, 342.

Morrison, F. O. (1948). Know the flies to control them. *Pests*, **16** (6), 9.

Patten, B. M. (1916). The changes of the blowfly larva's photosensitivity with age. *J. exp. Zool.* **20**, 585.

Paul, J. R. & Trask, J. D. (1942). Occurrence and recovery of the virus of infantile paralysis from sewage. *Amer. J. publ. Hlth*, **32**, 235.

Paul, J. R., Trask, J. D., Bishop, M. B., Melnick, J. L. & Casey, A. E. (1941). The detection of poliomyelitis virus in flies. *Science*, **94**, 395.

Potgieter, J. T. (1945). Blowflies and flies in towns. *Fmg S. Afr.* **20** (226), 47.

Sabin, A. B. & Ward, R. (1941). Flies as carriers of poliomyelitis virus in urban epidemics. *Science*, **94**, 590.

Thomsen, M. & Hammer, O. (1936). The breeding medium of some common flies. *Bull. ent. Res.* **27**, 559.

Trask, J. D. & Paul, J. R. (1943). The detection of poliomyelitis virus in flies collected during epidemics of poliomyelitis. 2. Clinical circumstances under which flies were collected. *J. exp. Med.* **77**, 545.

Trask, J. D., Paul, J. R. & Melnick, J. L. (1943). The detection of poliomyelitis virus in flies collected during epidemics of poliomyelitis. 1. Methods, results, and types of flies involved. *J. exp. Med.* **77**, 531.

Wardle, R. A. (1927). The seasonal frequency of Calliphorine blow-flies in Great Britain. *J. Hyg., Camb.*, **26**, 441.

Wardle, R. A. (1930). Significant variables in the blowfly environment. *Ann. appl. Biol.* **17**, 554.

(*Received* 10 *October* 1950)

STUDIES ON THE MECHANISM OF INSECTICIDAL ACTION OF ORGANO-PHOSPHORUS COMPOUNDS WITH PARTICULAR REFERENCE TO THEIR ANTI-ESTERASE ACTIVITY

By K. A. LORD and C. POTTER

Rothamsted Experimental Station, Harpenden, Herts

(With 1 Text-figure)

The preparation of an extract of the mealworm larvae, *Tenebrio molitor* L. which hydrolyses ethyl butyrate and *o*-nitrophenyl acetate, but not acetylcholine, is described. The inhibition of this esterase by TEPP-containing materials and parathion was determined.

An enzyme that hydrolysed *o*-nitrophenyl acetate and was inhibited by a TEPP-containing material was demonstrated in the five other insect species used.

The relative toxicities as contact insecticides to adult *Tribolium castaneum* Hbst. of ten samples of TEPP-containing materials were compared with their relative activities as esterase inhibitors. There was not an exact quantitative correlation between TEPP content estimated chemically, insecticidal activity and anti-esterase activity; but the correlation was sufficient to suggest interdependence of these factors.

Eggs of *Diataraxia oleracea* L. and *Ephestia kühniella* Zell, were shown to contain an enzyme that hydrolysed *o*-nitrophenyl acetate and was inhibited by the TEPP-containing materials. This enzyme was present in eggs less than 24 hr. old, i.e. before there was any visible signs of development. The TEPP was shown to be toxic to these eggs and in high concentrations kills at an early stage of development before differentiation of the nervous system. This, in conjunction with the other evidence, suggests that esterases other than the choline-esterase of the nervous system are important when considering the toxic action of these compounds.

Comparison of the anti-esterase activity and toxicity of parathion and TEPP-containing materials as insecticides showed that although the TEPP materials were the more potent enzyme inhibitors, parathion was the more potent contact insecticide to five species of insects. This appears to be due to the relative instability of TEPP. The study of the rates of action of the two poisons applied at different concentrations supports this view.

Introduction

The organo-phosphorus compounds E605 or parathion, hexaethyl tetraphosphate (HETP) and tetraethyl pyrophosphate (TEPP) are now widely used as insecticides. Workers in the U.S.A. (Hall & Jacobson, 1948*a*; Dvornikoff & Morrill, 1948) have published data indicating that the principal insecticidally active constituent in both HETP and TEPP is tetraethyl pyrophosphate; these materials are easily hydrolysed.

E605 and parathion are synonyms for insecticides which have *O*:*O*-diethyl-*O*-(*p*-nitrophenyl) thiophosphate as their main active constituent. This material is relatively stable to hydrolysis.

All these materials have a high mammalian toxicity which is generally attributed to their activity as anti-choline esterase agents. By analogy it was assumed that they might inhibit the choline esterases in insects and some work has been published on their activity in this connexion (Chadwick & Hill, 1947; Metcalf, 1948; Metcalf & March 1949).

The work described in this paper started as an attempt to investigate further the anti-esterase activity of these compounds. The insect preparations first studied failed to show choline esterase activity and the anti-choline esterase activity was not therefore confirmed. The preparations, however, showed considerable activity in hydrolysing ethyl butyrate although not acetylcholine; this activity was strongly inhibited by the organo-phosphorus compounds under study. The work was then extended first to obtain some data on the distribution of esterase and to determine whether there was any correlation between the anti-esterase activity, content of tetraethyl pyrophosphate and insecticidal activity. Some correlation was found. The investigation later included *O*:*O*-diethyl-*O*-(*p*-nitrophenyl) thiophosphate (parathion) which shows higher insecticidal activity weight for weight than the tetraethyl pyrophosphate-containing materials, although the latter are the more potent inhibitors of both insect and mammalian choline esterase activity (Dubois & Mangun, 1947; Dubois, Doull, Salerno & Coon, 1949; Metcalf & March, 1949), and they have also been shown to be more effective inhibitors of the esterase activity of our insect preparations. Work carried out on the speed of action of insecticides has, however, suggested an explanation of these effects. Additional experiments on ovicidal action are thought to provide an indication of the importance of the activity of these compounds as inhibitors of esterases other than choline esterase, in their mechanism of toxic action.

The work is not exhaustive but it is thought to provide some information on the possibility of using the estimation of anti-esterase activity as a measure of the insecticidal activity of these compounds and data on the mechanism of their action and some factors likely to affect it.

§ 1. The occurrence of esterase in insects and the effect of organo-phosphorus inhibitors

(*a*) *Preparation of the esterase and estimation of activity*

Preliminary tests with preparations of whole *Tribolium castaneum* Hbst. and *Tenebrio molitor* L. failed to show any activity in hydrolysing of acetylcholine. It should be pointed out that no persistent attempt was made to prepare an extract hydrolysing acetylcholine since these preparations were found to have considerable esterase activity in hydrolysing ethyl butyrate and *o*-nitrophenyl acetate. It is possible that with a different technique these insects may prove a satisfactory source of choline esterase.

The following technique of preparation was used for the quantitative studies

on esterase activity. About 30 g. of larvae of the mealworm *Tenebrio molitor* were minced with 200 ml. of ice cold water in a Waring blender. The resulting suspension was centrifuged and the supernatant liquid was decanted off. The clear liquid was cooled to 0° C. and treated with half its volume of ice-cold ethyl alcohol. This mixture was centrifuged, the solids separating were rejected and the clear liquid retained for use.

No measurable loss of activity of the extracts occurred over a period of a week.

The esterase activity of the preparation was determined by measuring the rate of liberation of *o*-nitrophenol from *o*-nitrophenyl acetate. The amount liberated in a given time at 25° C. was measured colorimetrically using a Unicam diffraction grating spectrophotometer (model S.P. 350) set to measure absorption at a wave length of 400 mμ. Allowance for non-enzymic hydrolysis was made by incubating the substrate under the conditions of the experiment in the absence of enzyme.

(*b*) *Estimation of anti-esterase activity*

The anti-esterase activity of the various preparations was tested at 25° C. using a colorimetric technique similar to that described by Jansen, Nutting & Balls (1948).

Two ml. of the esterase preparation, prepared as described above, was mixed with 200 ml. of 0·1 M-phosphate buffer pH 6·5 at 25° C. 20 ml. aliquots were measured out into the spectrophotometer tubes and each portion was treated with 0·2 ml. of inhibitor solution in ethylene glycol monoethyl ether. The mixture was allowed to stand for 20 min. at 25° C. and then 0·4 ml. of an ethanol solution of the substrate (*o*-nitrophenyl acetate) was added. The final concentration of *o*-nitrophenyl acetate was 0·002 M.

The percentage inhibition when plotted against log concentration gave an S-shaped curve which was approximately linear over the middle range. The concentration to give 50% inhibition (ID50) was determined graphically. The

TABLE 1. *Inhibitory powers of TEPP-containing materials and parathion to esterase* Tenebrio molitor

Sample no.	TEPP content (% w/v)	7+log ID50	Concentration $ID50 \times 10^7$ (%)	Relative activity
1	6·2	2·12	130	6
2	11·6	1·64	44	18
3	11·8	1·72	53	15
4	15·0	1·50	32	25
6	20·2	1·30	20	40
7	28·0	1·28	19	40
Parathion	—	1·68	48	16
4	15·0	1·00	10	25
5	18·2	0·90	7·9	32
7	28·0	0·80	6·3	40
8	40·2	0·76	5·8	44
9	63·5	0·50	3·2	79
10	75·1	0·40	2·5	100

results of tests of a number of samples of material containing different amounts of TEPP and of a sample of parathion, are shown in Table 1.

Using this technique and the larvae of *T. molitor* as the source of enzyme, an examination was made of the correlation between inhibition by a number of TEPP-containing materials and their insecticidal activity, the results of which are set out in Table 5, §2.

(*c*) *Occurrence of esterase in insects*

Qualitative tests were carried out on a number of instars and species of insects to determine whether extracts from them showed activity similar to that of *T. molitor* extracts and thus to obtain some information on how widely this type of activity was distributed in insects. At the same time tests were made to determine whether the hydrolysis was inhibited by TEPP-containing materials.

The insect material was finely ground with 4 ml. of water and centrifuged. One ml. of the homogenate was added to 10 ml. of 0·1 M-phosphate buffer pH 6·5, and served as a blank to compensate for the natural colour of the preparation.

A second 1 ml. portion of the preparation was added to 10 ml. of phosphate buffer containing 0·1 ml. of ethanol, this was then treated with 0·2 ml. of an ethanol solution of *o*-nitrophenyl acetate to give a final concentration of *o*-nitrophenyl acetate of 0·002 M.

Table 2. *Presence of esterase activity in various insect preparations and its inhibition by a TEPP-containing material (sample no. 4, 15% TEPP)*

Insect species and stage	Quantity of insects	Presence of esterase	Inhibitory effect of TEPP-containing material (15% TEPP)	
			15×10^{-4}%	15×10^{-6}%
Diataraxia oleracea (tomato moth) eggs	500 individuals	Present	Complete inhibition	Partial inhibition
Ephestia kühniella (Mediterranean flour moth) eggs	0·06 g.	Present	Complete inhibition	—
Diataraxia oleracea (tomato moth) 5th instar larvae	1 individual	Present	Complete inhibition	Partial inhibition
Plutella maculipennis (diamond back moth) last instar larvae	6 individuals	Present	Complete inhibition	Partial inhibition
Macrosiphum euphorbiae (potato aphis) a.v.p.f.*	150 individuals	Present	Complete inhibition	—
Acyrthosiphon pisum (pea aphis) a.v.p.f.*	170 individuals	Present	Complete inhibition	Partial inhibition

* Apterous viviparous parthenogenetic females.

In a third and fourth tube the homogenate was allowed to stand for 20 min. in the presence of a sample of HETP (no. 4) with an estimated TEPP content of approximately 15%. At the end of 20 min. the colour developed in all the tubes was observed and from the data obtained the esterase activity of the preparation and its inhibition by the TEPP-containing sample was deduced.

All the activity tests were carried out in a constant temperature water bath at 25° C.

Table 2 shows the data obtained.

Inspection of Table 2 shows that esterase activity is found in both eggs and active stages covering a range of five different species of insects. Furthermore, it appears that in every instance the esterase activity is inhibited by a TEPP-containing material. It seemed, therefore, that the toxic action of the TEPP-containing materials might be due, at least in part, to their capacity to inhibit this activity. It was also thought that this might be particularly important when considering ovicidal action, where, in the early stage of development, no specialized nervous system exists and therefore, choline esterase of the nervous system if present at all, might not be essential.

If inhibition of an esterase forms an important part of the insecticidal action of these organo-phosphorus compounds it seems reasonable to suppose that there would be some correlation between their toxicity as insecticides and anti-esterase activity. Experiments described in the following section, were carried out to obtain data on this point.

§2. The relationship between TEPP content, anti-esterase activity and contact insecticidal action of TEPP-containing materials

A series of tests of the contact insecticidal activity of ten samples containing different amounts of tetraethyl pyrophosphate were carried out. An estimate was made of the toxicity to adults of the flour beetle, *Tribolium castaneum* Hbst., of all the materials as contact insecticides. Six of the TEPP-containing materials were also tested against apterous viviparous parthenogenetic females of the potato aphis *Macrosiphum euphorbiae* Thos., and the fully grown larvae of *Plutella maculipennis* Curt., the diamond back moth.

The apparatus and technique used were those described by Potter (1941). The insects were sprayed throughout in 9 cm. dishes using tricoline circles as substrate. The spray deposit was arranged to be 400–500 mg. per dish in each experiment. Three replicates were sprayed at each concentration and at least fifteen insects were used per replicate. After treatment the insects were kept in the basement room which normally maintains a temperature between 55 and 65° F. and a relative humidity between 40 and 68%.

The m.l.c.'s and the relative potencies of the materials were derived from probit regression equations calculated from the data.

A series of tests were required to obtain these data and they are set out in Tables 3

and 4 which give the relative potency, the log M.L.C. and the slope of the regression lines together with their standard errors.

TABLE 3. *Toxicity of TEPP-containing materials to* Tribolium castaneum *as direct spray*

Test	Sample no.	Log (LD 50)	Slope	Relative potency
1	1	$\bar{1}\cdot235 \pm 0\cdot043$	$2\cdot71 \pm 0\cdot33$	18
	2	$\bar{1}\cdot008 \pm 0\cdot032$	$2\cdot89 \pm 0\cdot28$	30
	3	$\bar{1}\cdot043 \pm 0\cdot036$	$2\cdot71 \pm 0\cdot28$	28
	4	$\bar{2}\cdot933 \pm 0\cdot023$	$2\cdot82 \pm 0\cdot30$	36
	6	$\bar{2}\cdot853 \pm 0\cdot031$	$3\cdot10 \pm 0\cdot28$	44
	7	$\bar{2}\cdot644 \pm 0\cdot028$	$3\cdot47 \pm 0\cdot30$	70
2	4	$\bar{2}\cdot535 \pm 0\cdot032$	$3\cdot00 \pm 0\cdot31$	36
	8	$\bar{2}\cdot324 \pm 0\cdot023$	$3\cdot71 \pm 0\cdot27$	59
	10	$\bar{2}\cdot094 \pm 0\cdot028$	$2\cdot93 \pm 0\cdot24$	100
3	4	$\bar{2}\cdot667 \pm 0\cdot030$	$2\cdot24 \pm 0\cdot24$	36
	5	$\bar{2}\cdot706 \pm 0\cdot030$	$2\cdot40 \pm 0\cdot25$	33
4	4	$\bar{2}\cdot718 \pm 0\cdot042$	$1\cdot99 \pm 0\cdot29$	36
	5	$\bar{2}\cdot733 \pm 0\cdot041$	$2\cdot55 \pm 0\cdot28$	35
5	4	$\bar{2}\cdot428 \pm 0\cdot030$	$3\cdot49 \pm 0\cdot36$	36
	9	$\bar{3}\cdot871 \pm 0\cdot029$	$3\cdot47 \pm 0\cdot37$	100
6	4	$\bar{2}\cdot360 \pm 0\cdot031$	$2\cdot52 \pm 0\cdot22$	36
	9	$\bar{3}\cdot979 \pm 0\cdot032$	$3\cdot08 \pm 0\cdot29$	87
	10	$\bar{3}\cdot987 \pm 0\cdot065$	$3\cdot47 \pm 0\cdot38$	85

TABLE 4. *Toxicity of TEPP-containing material to various insects as direct sprays*

Sample no.	Log (LD 50)	Slope	Relative potency
	Macrosiphum euphorbiae		
1	$\bar{2}\cdot163 \pm 0\cdot029$	$6\cdot00 \pm 0\cdot97$	22·6
2	$\bar{2}\cdot051 \pm 0\cdot037$	$4\cdot21 \pm 0\cdot54$	29·2
3	$\bar{3}\cdot922 \pm 0\cdot034$	$4\cdot62 \pm 0\cdot67$	39·4
4	$\bar{3}\cdot961 \pm 0\cdot033$	$4\cdot87 \pm 0\cdot73$	36·0
6	$\bar{3}\cdot805 \pm 0\cdot039$	$3\cdot56 \pm 0\cdot43$	51·6
7	$\bar{3}\cdot618 \pm 0\cdot040$	$3\cdot24 \pm 0\cdot39$	79·5
	Plutella maculipennis		
1	$\bar{1}\cdot230 \pm 0\cdot042$	$3\cdot82 \pm 0\cdot62$	20·0
2	$\bar{1}\cdot059 \pm 0\cdot054$	$2\cdot56 \pm 0\cdot38$	25·6
3	$\bar{1}\cdot009 \pm 0\cdot053$	$2\cdot59 \pm 0\cdot38$	33·2
4	$\bar{2}\cdot976 \pm 0\cdot051$	$2\cdot75 \pm 0\cdot41$	36·0
6	$\bar{1}\cdot019 \pm 0\cdot041$	$4\cdot28 \pm 0\cdot74$	32·6

All the materials could not be tested at one time on *Tribolium castaneum* and six tests were carried out. Sample no. 4 was included in all the tests as a basis for comparison. Some samples were tested more than once and did not give identical relative toxicities in the different tests although there were no large differences. In these cases the different relative potencies were averaged and the average figures used

for the purpose of comparing the insecticidal activity with anti-esterase activity is set out in Table 5.

The ID 50 of each of the ten TEPP samples as inhibitors of the esterase activity of a preparation from the larvae of *Tenebrio molitor* was estimated by the technique described in § 1.

Table 5 shows these data compared with the data on relative potency obtained on the toxicity trials and the TEPP content of the samples as estimated by the method of Hall & Jacobson (1948*b*).

TABLE 5. *Correlation between TEPP content, contact insecticidal activity and anti-esterase capacity of TEPP-containing materials to three insect species*

			Relative potency as contact insecticides		
Sample no.	TEPP content (% w/v)	Relative anti-esterase activity	*T. castaneum* adults	*M. euphorbiae* apterous viviparous parthenogenetic females	*P. maculipennis* final instar larvae
1	6·2	6	18	22·6	20·0
2	11·6	18	30	29·2	25·6
3	11·8	15	28	39·4	33·2
4	15·0	25	36	36	36
5	18·2	32	34	—	—
6	20·2	40	44	51·6	32·6
7	28·0	40	70	79·5	—
8	40·2	44	59	—	—
9	63·5	79	93·5	—	—
10	75·1	100	92·5	—	—

From Table 5 it will be seen that while there is not a strict quantitative correlation between TEPP content, contact insecticidal activity and esterase inhibition, it is sufficiently close to suggest interdependence.

Thus, while some anomalies exist, the table shows that in general an increase in content of TEPP results in an increase of anti-esterase activity and an increase in insecticidal activity. Chadwick & Hill (1947) studied the relationship between the anti-choline esterase activity and toxicity by injection of hexaethyl tetraphosphate to cockroaches *Periplaneta americana* L. They found that the correlation between toxicity and enzyme inhibition was less satisfactory for HETP and physostigmine than for *d*-isopropyl fluorophosphate (DFP). Cockroach nerve cords were used as the source of choline esterase. They concluded that with DFP the results strongly indicate that the anti-choline esterase activity will account for at least the major share of its toxic action and suspect that a similar relationship exists for physostigmine and HETP. They add, however, that the possibility of additional toxic mechanisms for any of the agents cannot be excluded on the basis of their data.

The experiments described here showed a rather closer correlation between

TEPP content, determined by chemical assay, and esterase inhibition than between TEPP content and contact toxicity. These results, however, do indicate that the action of organo-phosphorus compounds on esterases other than choline esterase may at least under some circumstances, prove to be important, when considering insecticidal activity.

In order to obtain some additional evidence on the problem of whether inhibition of the esterase under consideration is an important part of the mechanism of toxic action, experiments were carried out on insect eggs. This work is described in the next section.

§3. The ovicidal action of TEPP-containing materials and its relationship to anti-esterase activity

The presence in eggs of *Diataraxia oleracea* L., the tomato moth, of an enzyme capable of hydrolysing *o*-nitrophenyl acetate was established as described in §1 and an esterase with similar properties was also found to be present in eggs of *Ephestia kühniella* Zell, the Mediterranean flour moth. The esterase from both sources was inhibited by TEPP-containing materials. A further experiment on eggs of *Diataraxia oleracea* L. showed that the esterase activity was present in eggs less than 24 hr. old before there was any visible organization. The esterase activity of the young eggs was also inhibited by a TEPP-containing material.

The TEPP-containing materials have been said to be lacking ovicidal activity but they should not be entirely inactive if this esterase plays an important part in metabolism. While it is recognized that choline esterases occur in tissues other than the nervous system, it seems to have been generally supposed that the toxicities of the compounds under consideration are probably due to their inducing an accumulation of acetylcholine in the nervous system. It seems unlikely that choline esterase of the nervous system would be essential, at least in the earlier stages of the development of the eggs, before the differentiation of a nervous system. It seemed, therefore, that if the TEPP-containing materials were shown to be capable of killing eggs at an early stage of development, this would be evidence that their anti-choline esterase activity was not necessarily the only way in which they could exert a toxic action, and would provide evidence indicating that their action on the esterase activities described above, forms an important part of their toxic action.

The contact ovicidal effect of one of the samples was therefore tested on the eggs of both *Diataraxia oleracea* and *Ephestia kühniella*. The results are set out in Table 6. It will be seen from this table that the sample is toxic to both species of egg although the concentrations required to kill are rather high. A note was made of the stage of development, as judged by inspection under the microscope, at which the eggs had been killed and it was found that whereas at the lower dosage a high percentage of eggs formed highly developed embryos before death; in the higher dosages it appeared that some of the eggs were killed before any visible embryonic

development had taken place. Some further experiments were carried out with eggs of *Diataraxia oleracea* in which measured drops approximately 0·0004 c.c. of concentrated HETP containing 15% tetraethyl pyrophosphate were placed in individual eggs, and the results compared with those on untreated eggs. Two series of tests were made. In the first the eggs received no treatment except the application of the drop of tetraethyl pyrophosphate. In the second series both control and treated eggs were first sprayed with a 0·5% w/v solution of Lissapol N which was subsequently allowed to dry and then the poison treatments applied. Each series consisted of three experiments. In the first, the eggs were placed on a plain glass surface so that no absorption of the drop of poison took place, in the second the eggs were placed on tricolene which acted as a mild absorbent and in the third the eggs were placed on filter paper which acted as a strong absorbent. After treatment the eggs were placed in a constant temperature cabinet at 75° F.

After the controls had hatched, the unhatched eggs of both controls and treated batches were dissected and examined for visible development. The results are set out in Table 7. It appears that it is necessary to treat the eggs within 24 hr. of oviposition if they are to be killed before any visible development occurs. The first visible sign noted was the occurrence of eye spots.

From the data given in Tables 6 and 7 it is reasonable to assume that TEPP-containing materials can exert an ovicidal action and can kill eggs at an early stage of development before the nervous system is fully differentiated.

TABLE 6. *Toxicity of sample 7 (TEPP content 28% w/v) to the eggs of* Diataraxia oleracea *(Tomato moth) and* Ephestia kühniella *(Mediterranean flour moth)*

Diataraxia oleracea				*Ephestia kühniella*			
Conc. of sample 7 (% w/v)	No. of eggs in test	No. of eggs killed	percentage kill	Conc. of sample 7 (% w/v)	No. of eggs in test	No. of eggs killed	percentage kill
0·625	62	62	100	5·0	22	22	100
0·3125	57	52	91	2·5	26	25	96
0·1562	61	46	75	1·25	39	35	90
0·0781	42	18	42	0·625	34	16	47
0·0391	58	24	41	0·3125	33	14	42
Control	—	—	—	0·156	33	14	42
				0·078	39	11	28
				Control	56	9	—

Probit regression equation:

$Y = 5·4375 + 2·6024\ (x - 3·1700)$

$b = 2·60 \mp 0·12$

$m = \log LD50 \times 10^4 = 3·0082 \mp 0·050$

LD50 = 0·1008% w/v

Probit regression equation:

$Y = 5·0572 + 1·8127\ (x - 3·7150)$

$b = 1·81 \mp 0·35$

$m = \log LD50 \times 10^4 = 3·693 \mp 0·0634$

LD50 = 0·494% w/v

TABLE 7. *Mortality and inhibition of development of eggs of* Diataraxia oleracea *caused by measured drops of HETP containing approximately* 15% *tetraethyl pyrophosphate*

Size of drop per egg = 0·0004 c.c. approx. Age of eggs = less than 24 hr. Temperature after treatment, 75° F. Series 1: eggs given no treatment prior to application of the poison. Series 2: all eggs sprayed with 0·5% Lissapol N prior to application of the poison.

					Stage of development of unhatched eggs			
Series	Treatment	Substratum	Hatched	Un-hatched	Fully de-veloped	96–120 hr.	72–96 hr.	Unde-veloped
1	Control	Glass	19	1	—	—	—	1
	HETP	Glass	1	19	—	—	7	12
2	Control	Glass	13	6	—	1	—	5
	HETP	Glass	0	19	—	—	5	15
1	Control	Tricolene	19	1	—	—	1	—
	HETP	Tricolene	0	20	—	2	—	18
2	Control	Tricolene	19	1	—	1	—	—
	HETP	Tricolene	0	20	—	—	—	20
1	Control	Filter-papers	20	0	—	—	—	—
	HETP	Filter-papers	0	17	—	1	2	14
2	Control	Filter-papers	17	3	—	—	—	3
	HETP	Filter-papers	0	19	—	—	—	19

§4. THE ANTI-ESTERASE ACTIVITY OF PARATHION AND A COMPARISON OF ITS ACTION WITH THAT OF TEPP-CONTAINING MATERIALS

In view of the apparent correlation between the anti-esterase activity of the TEPP-containing materials and their insecticidal action and the known anti-choline esterase activity of parathion it appeared desirable to determine whether *O*:*O*-diethyl-*O*-(*p*-nitrophenyl) thiophosphate (parathion) inhibited the same enzyme preparation and to compare its behaviour with that of TEPP-containing materials.

The results of the contact insecticidal trials to compare parathion and TEPP-containing materials are set out in Table 8.

The sample of parathion was tested for its capacity to inhibit the esterase preparation from *Tenebrio molitor* as previously described. The anti-esterase activity was compared with that of TEPP-containing materials and the relationship between esterase inhibition and insecticidal activity to a number of insect species is set out in Table 9.

From Table 9 it will be seen that whereas the capacity to inhibit esterase activity of the two TEPP-containing materials is about 2·5 times that of the parathion, the contact insecticidal action of the latter ranges from about 6 to 125 times that of TEPP-containing materials, dependent on the species and instar used as test subject. It is interesting to note that while the difference in relative potency between parathion and TEPP is high for the eggs of *Ephestia kühniella*, it is not so great for eggs of

TABLE 8. *Toxicity of parathion and TEPP-containing materials to various insects as direct sprays*

	log (LD 50)	Slope	Relative potency
Plutella maculipennis larvae			
Sample 8	$\bar{2}\cdot543 \pm 0\cdot049$	$2\cdot95 \pm 0\cdot47$	1
Parathion	$\bar{3}\cdot739 \pm 0\cdot035$	$4\cdot77 \pm 0\cdot73$	6·3
Phaedon cochleariae adults			
Sample 8	$\bar{2}\cdot184 \pm 0\cdot036$	$4\cdot11 \pm 0\cdot54$	1
Parathion	$\bar{4}\cdot789 \pm 0\cdot028$	$6\cdot88 \pm 1\cdot11$	25
Tribolium castaneum adults			
Sample 8	$\bar{2}\cdot324 \pm 0\cdot023$	$3\cdot71 \pm 0\cdot27$	1
Parathion	$\bar{4}\cdot890 \pm 0\cdot020$	$5\cdot22 \pm 0\cdot44$	27
Diataraxia oleracea eggs			
Sample 7	$\bar{1}\cdot004 \pm 0\cdot050$	$2\cdot60 \pm 0\cdot12$	1
Parathion	$\bar{3}\cdot536 \pm 0\cdot051$	$2\cdot78 \pm 0\cdot25$	29
Ephestia kühniella eggs			
Sample 7	$\bar{1}\cdot599 \pm 0\cdot096$	$1\cdot81 \pm 0\cdot35$	1
Parathion	$\bar{3}\cdot693 \pm 0\cdot063$	$2\cdot90 \pm 0\cdot34$	125

TABLE 9. *A comparison between the relative anti-esterase activity of parathion (O:O-diethyl-O-(p-nitrophenyl) thiophosphate) and two samples of TEPP-containing materials and their relative contact insecticidal activity to different species and instars of insects*

	Parathion	Sample no. 7 TEPP content (28 % w/v)	Sample no. 8 TEPP content (40·25 % w/v)
Relative anti-esterase activity	1	2·4	2·6
Relative toxicity *Tribolium castaneum* Hbst. adults	27	—	1
Relative toxicity *Phaedon cochleariae* Fab. adults	25*	—	1*
Relative toxicity *Plutella maculipennis* Curt. larvae	6*	—	1*
Relative toxicity *Diataraxia oleracea* L. eggs	29	1	—
Relative toxicity *Ephestia kühniella* Zell. eggs	125	1	—

* Probit regression lines not parallel: relative toxicity measured at the M.L.C.

Diataraxia oleracea. Thus, ovicidal action of TEPP-containing materials is relatively not much inferior to their toxicity to active stages when compared with parathion which is recognized as an ovicide.

If TEPP and parathion do act on the same system and are toxic by virtue of their action on a choline esterase or another esterase, it might be expected that TEPP would be the more potent insecticide, since the available evidence indicates that it is the more potent enzyme inhibitor (Metcalf & March, 1949; Dubois & Mangun, 1947; Dubois *et al.* 1949).

When both were tested as contact insecticides however, parathion was found to be more potent than TEPP under the conditions of the experiments.

It is possible to provide an explanation for this apparent contradiction by taking into account both the ease of hydrolysis of TEPP relative to parathion and the need for the poisons to penetrate to, and accumulate at the site of action. A study of the relative rates of action of the two poisons at various concentrations provides some evidence in support of this.

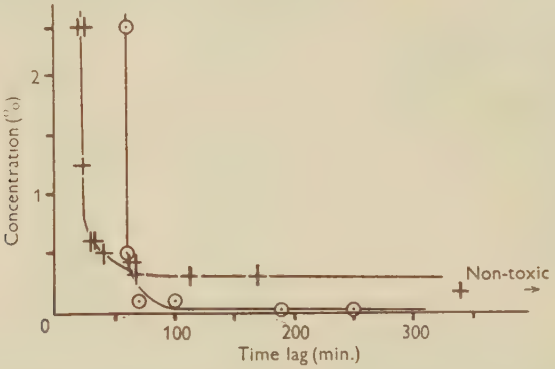


Fig. 1. The effects of concentration of organo-phosphorus insecticides applied as dusts to *Tribolium castaneum* on the time-lag between application and the beginning of the rise of oxygen uptake. Key: ⊙, parathion; +, sample no. 4 (15% TEPP).

Fig. 1 shows a comparison of the effect of concentrations of parathion and sample no. 4 (15% TEPP) on the time lag between application and the rise in oxygen uptake of adult *Tribolium castaneum*. The technique used for making the comparisons was that described by Lord (1950*a*). The experiments were carried out at a temperature of 25° C. and approximately 80% relative humidity.

The data show that at high concentrations TEPP acts more rapidly than parathion. Under these conditions it is reasonable to assume that the poison is entering the cuticle and accumulating at the site of action sufficiently rapidly to produce a reaction before much hydrolysis can occur; the greater rapidity of action of TEPP under these conditions may be explained by the fact that it is a more potent enzyme inhibitor than parathion, although other factors such as penetration may also affect its speed of action.

After a certain time, however, a higher concentration of TEPP than parathion is required to produce the reaction in a given time. Moreover, there is a critical concentration below which TEPP is non-toxic whilst parathion appears to kill at very much lower dilutions, although the toxic effects may be delayed for a considerable period of time (Lord, 1950*b*).

It may be argued in the case of TEPP that at concentrations where the poison is entering the cuticle in small amounts and a considerable time elapses before effective quantities accumulate at the site of action, hydrolysis can occur to a significant extent, thus further delaying accumulation at the site of action and further

extending the reaction of time. Under these conditions there would be a reduction in the apparent or observed toxicity of the poison since some of it is destroyed before it can become effective. (In the usual toxicity trials the concentrations used are limiting and these factors presumably come into play to reduce the apparent toxicity.) Owing to the greater stability of parathion, the longer time required for a given amount to accumulate at the site of action does not affect its toxicity in the same way.

Our thanks are due to Messrs Albright and Wilson for supplying various samples of TEPP-containing materials and parathion, and to Dr B. A. Kilby for a highly purified sample of TEPP.

REFERENCES

Chadwick, L. E. & Hill, D. L. (1947). Inhibition of cholinesterase by diisopropyl fluorophosphate, physostigmine and hexaethyl tetraphosphate. *J. Neurophysiol.* **10**, 235.

Dubois, K. P., Doull, J., Salerno, P. R. & Coon, J. M. (1949). Studies on the toxicity and mechanism of action of *p*-nitrophenyl diethyl thionophosphate (Parathion). *J. Pharmacol.* **95**, 79.

Dubois, K. P. & Mangun, G. H. (1947). Effect of hexaethyl tetraphosphate on cholinesterase *in vitro* and *in vivo*. *Proc. Soc. exp. Biol., N.Y.*, **64**, 137.

Dvornikoff, M. N. & Morrill, H. L. (1948). Determination of tetraethyl pyrophosphate in mixtures of ethyl phosphate esters. *Analytical chem.* **20**, 935.

Hall, S. A. & Jacobson, M. (1948*a*). Hexaethyl tetraphosphate and tetraethyl pyrophosphate. *Industr. Engng Chem.* **40**, 694.

Hall, S. A. & Jacobson, M. (1948*b*). Chemical assay of tetraethyl pyrophosphate. *Agric. Chem.* **3**, 30.

Jansen, E. F., Nutting, M. D. F. & Balls, A. K. (1948). The reversible inhibition of acetylesterase by di-isopropyl fluorophosphate and tetraethyl pyrophosphate. *J. biol. Chem.* **175**, 975.

Lord, K. A. (1950*a*). The effect of insecticides on respiration. II. The effects of a number of insecticides on the oxygen uptake of adult *Tribolium castaneum* Hbst. at 25° C. *Ann. appl. Biol.* **37**, 105.

Lord, K. A. (1950*b*). The effects of concentration of poison and the amount applied on the toxicity of insecticidal dusts to adult *Tribolium castaneum* Hbst. *Ann. appl. Biol.* **37**, 123.

Metcalf, R. L. (1948). The mode of action of organic insecticides. *Chem.-Biol. Coordination Center*, Rep. no. 1, pp. 71–7. Nat. Res. Coun., Washington.

Metcalf, R. L. & March, R. B. (1949). Studies of the mode of action of parathion and its derivatives and their toxicity to insects. *J. econ. Ent.* **42**, 721.

Potter, C. (1941). A laboratory spraying apparatus and technique for investigating the action of contact insecticides, with some notes on suitable test insects. *Ann. appl. Biol.* **28**, 142.

(*Received* 10 *August* 1950)

INSECTICIDAL-ACTION STUDIES WITH BISDIMETHYLAMINOPHOSPHONOUS ANHYDRIDE CONTAINING ^{32}PHOSPHORUS

By W. A. L. DAVID

Agricultural Research Council Unit of Insect Physiology, University of Cambridge

Bisdimethylaminophosphonous anhydride containing ^{32}P has been used to study the absorption of this systemic insecticide into plants and its action on aphids.

The radio-anhydride is absorbed by the roots of broad beans placed in culture solutions containing it. The level of radio-activity in the plant increases as the solution is absorbed and is higher in the washed roots than in the rest of the plant. The activity of the remaining culture solution increases as more is absorbed showing that the roots selectively reject the radio-anhydride. The material is absorbed more slowly from soil than from sand. In both cases the concentration per gram of tissue is highest in the leaves on the middle part of the stem.

By introducing the insecticide by the cut tap-root technique it can be shown that about 50% of the radio-anhydride is decomposed within the plant in 8 days. The concentration of undecomposed anhydride in the plant necessary to give a complete kill of *Aphis fabae* Scop. is about 50 mg./kg. of beans. Dead aphids were found to contain about 15–20 mg./kg. of radioactive material calculated on the assumption that it was undecomposed anhydride. The honey-dew of aphids feeding on treated plants is also radioactive.

Absorption and translocation of radioactive material occurs following the application of the radio-anhydride to the leaves of the broad bean, cabbage, hop, pea and strawberry. In the broad bean radioactive material can be detected within the leaf a few hours after it has been applied to the surface. In all plants there is evidence that radioactive material is translocated to untreated parts. Much more is translocated to leaves younger than those treated than to leaves older than those treated. In favourable cases, where a large number of leaves on the plant are treated, where the plant holds a large quantity of the anhydride applied or where a heavy dose is given, either by repeated treatments or the use of high concentrations, it is found that insecticidal quantities of what is presumed to be the anhydride are translocated to untreated young growing parts of the plants.

No measurable quantity of radioactive material is transpired by plants taking up the radio-anhydride by the roots.

Introduction

Bisdimethylaminophosphonous anhydride is one of the materials discovered by Schrader and Kükenthal that has been shown to possess systemic insecticidal properties. Several workers besides Schrader (1947) have shown that it is absorbed by the roots of plants (Bennett & Martin, 1947; Bennett, Martin, Stringer & Woodcock, 1948; Ripper, Greenslade & Hartley, 1950), and there is now general agreement that it is also translocated following applications to the leaves of growing plants though, in certain cases, this may be difficult to demonstrate (David & Kilby, 1949; David & Gardiner, 1951).

The only method used for following the uptake and translocation of the material in plants to date has been to observe its insecticidal action. This ceases to be a satisfactory procedure when the sensitivity of the insects to the poison or the capacity of the plant to absorb and translocate the material are unknown. It was a difficulty of this nature encountered in attempts to demonstrate systemic activity following applications made to broad-bean leaves that led to the present experiments with the radio-anhydride (David, 1950).

Materials and methods

The preparation of the radio-anhydride was first reported by Gardiner & Kilby (1949), and a detailed description of the methods employed is now available (Gardiner & Kilby, 1950). The activity of the solutions used in the experiments was seldom more than the equivalent of about 10 μc./l. The radioactivities of the samples were assayed on standard Geiger-Müller counting equipment maintained in a constant temperature room. A liquid-type counter (Veall, 1948) and a 10 min. counting period were used for almost all the assays. In the tables, where counts are given, the figures are corrected for background but not for the fact that this type of counter is only about 8–10% efficient for ^{32}P. This was, however, taken into account when determining the approximate absolute radioactivity of a solution.

The plants were kept in a greenhouse under the conditions described elsewhere, whilst being treated with the radio-anhydride (David & Gardiner, 1951). Most of the experiments were on broad beans, *Vicia faba* L. infested with *Aphis fabae* Scop., but some young cabbages infested with *Brevicoryne brassicae* L. and *Myzus persicae* Sul., young peas infested with *Acyrthosiphum onobrychidis* Fons., and strawberry plants infested with *Pentatrichopus fragaefolii* Cock., and newly sprouted hops sometimes infested with the bean or strawberry aphid were also employed.

In order to use the liquid-type counter already mentioned, it is necessary to extract the radioactive material from the plant. This can be done by boiling the chopped plant with sodium hydroxide solution. Preliminary experiments in which dilute solution of the anhydride were boiled alone and with equal volumes of sodium hydroxide solution showed that this treatment was unlikely to involve much loss of anhydride. There is, however, some loss of activity when the soda solutions are boiled down to less than half volume (Table 1).

Table 1. *Loss of radio-anhydride from boiled solutions*

	To half volume		To less than half volume	
Treatment given	Counts/min.	Percentage remaining	Counts/min.	Percentage remaining
None	598	100	642	100
Boiled/water	600	100	649	100
Boiled/2% NaOH	587	98	—	—
Boiled/10% NaOH	578	96·5	573	89

There appeared to be a greater tendency for the radio-anhydride to be lost when boiled in caustic soda than when boiled with water, and it seemed possible that it might be better to extract the chopped plant material with boiling water alone. The effect of adding an appreciable quantity of the inactive anhydride during the process, in order to depress the absorption losses of the anhydride was also tested. In these experiments the radio-anhydride was fed to the plants by the cut tap-root technique (David & Gardiner, 1951) and was totally absorbed in a few hours. The capsule containing the dose was washed into the plant extract before making up to volume. In the first experiment each plant was assayed separately, while in the second experiment two plants were assayed together. The boiling time was 0·5–1 hr., and water was added if too much evaporated.

Table 2. *Recovery of radioactive material from plants treated with the radio-anhydride*

Treatment, plants boiled with	Percentage recovered in Exp. 1	Exp. 2
Water	72·75	67
Anhydride solution, 0·2%	60·70	62
Sodium hydroxide solution, 8%	84·85	79

The values obtained with the 0·2% anhydride solution extract seem somewhat anomalous, but it can be seen that extraction and recovery of the anhydride is better with sodium hydroxide solutions than with water. A closer standardization of the time and rate of boiling during the extraction process than was adopted in these experiments seems desirable.

Any decomposition products of the anhydride formed in the plant may be radioactive if they contain phosphorus without being appreciably insecticidal. Measuring the radioactivity does not therefore indicate the amount of unchanged anhydride in the plant but the amount of anhydride plus radioactive decomposition products. There is evidence from insecticidal action studies that the rate of decomposition of the insecticidally active material in the plant, presumed to be the anhydride, is not very rapid. In the early stages of an experiment therefore the radioactivity can be taken as a good guide to the insecticidal content of the plant. But if a more precise measure of the amount of anhydride present is required, the procedure outlined by Hartley & Heath (1950) may be adopted. The plant material is weighed, chopped and boiled for 1 hr. with water to which some inactive anhydride has been added. The solution is cooled and made up to a known volume and this solution is assayed in a liquid-type counter. This gives the total amount of radioactive material extractable from the plant tissue. This solution is then heated to 80° C. for half an hour with enough solid sodium hydroxide to make it N/5. The anhydride is left unchanged but most, though possibly not all, decomposition products containing phosphorus will be converted into chloroform insoluble compounds. The solution is cooled,

made up to about 2 N with further quantities of sodium hydroxide and then extracted with chloroform. The anhydride partitions strongly in favour of the chloroform (about 97%) and can be assayed.

Results

Absorption by the roots from culture solutions

Bean plants placed in 0·05% (v/v) solutions of the anhydride become toxic to aphids in a day and on the third day the plants are entirely free of insects (David & Gardiner, 1951). The plants in the present experiments were allowed to absorb a solution of the radio-anhydride of the same concentration, and the radioactivity of each was measured after different periods in the solution. From the results given in Table 3 it may be seen that radioactive material passed into the three parts of the plant assayed. The level of activity increased steadily in all parts during the 7 days of the experiment. As might be expected, it was always higher in the washed roots than in the aerial parts of the plants.

Table 3. *The rate of accumulation of radioactive material by broad bean plants*

Plant nos.	Time sampled (hr.)	Root assay		Bean assay		Top assay	
		Counts/g.	mg./kg.	Counts/g.	Mg./kg.	Counts/g.	Mg./kg.
1	2·25	21·6	46·5	0·0	0·0	4·8	10·3
2		18·2	39·2	0·0	0·0	1·0	2·2
3	5·0	46·6	100	0·7	1·5	14·7	31·7
4		62·6	134	0·0	0·0	8·3	17·8
5	10	65·0	140	1·8	3·8	10·8	23·2
6		37·5	80·5	2·1	4·5	12·9	27·7
7	48	165	355	9·9	21·4	40·5	87·3
8		133	286	8·7	18·7	32·0	68·8
9	72	169	364	14·6	31·4	71·0	153
10		193	415	11·7	25·2	76·0	163
11	168	205	442	25·5	54·8	140	301
12		286	615	12·7	27·3	126	271

93 counts/min. = 0·2 mg. anhydride.

It was found that most of the aphids fell off the plants during the first and second days of the experiment. At this time the concentration of anhydride in the plant tissue, calculated on the assumption that none had decomposed, had reached the range of 60–100 mg./kg. previously found to be lethal to aphids (David & Kilby, 1949; David & Gardiner, 1951).

Experiments were planned to determine whether the anhydride is selectively absorbed or rejected by the roots of plants. In the broad bean it has been found that from a given volume of culture solution containing the anhydride, relatively more water than anhydride is absorbed. Thus even without allowing for the decay of radioactivity which occurs there is an increase in the activities of all solutions

Table 4. *The selective rejection of the anhydride by the roots of the broad bean*

(Obs. ct. = observed count. Corr. ct. = count corrected for radio-decay to day 0. Vol. abs. = cc. of solution absorbed by plants from the 100 cc. supplied. Gain or loss % = percentage increase or decrease in activity of solution.)

Days...		0	2				5				8			
Source	Conc. (%)	Obs. ct.	Obs. ct.	Corr. ct.	Vol. abs.	Activity gain or loss (%)	Obs. ct.	Corr. ct.	Vol. abs.	Activity gain (%)	Obs. ct.	Corr. ct.	Vol. abs.	Activity gain (%)
Plant	0·05	2550	2443	2718	12	18	2660	3502	36	49	2822	4360	53	82
			2448	2720	14	18	2643	3480	36	48	2828	4362	57	82
Plant	0·01	510	483	536	15	5	624	820	50	61	978	1510	76	196
			483	536	15	5	617	812	48	59	1056	1630	80	204
Plant	0·005	255	227	252	12	−1	255	336	41	32	306	473	60	86
			230	256	18	0·3	259	341	39	34	333	515	62	102
Standard	0·05	2550	2330	2550	—	—	1945	2550	—	—	1656	2550	—	—

Table 5. *The selective rejection of the anhydride by the roots of the broad bean*

(Abbreviations as in Table 4.)

Days...		0	1				2				4				6			
Source	Conc. (%)	Obs. ct.	Obs. ct.	Corr. ct.	Vol. abs.	Gain or loss (%)	Obs. ct.	Corr. ct.	Vol. abs.	Gain (%)	Obs. ct.	Corr. ct.	Vol. abs.	Gain (%)	Obs. ct.	Corr. ct.	Vol. abs.	Gain (%)
Plant	0·05	843	821	872	10	5	840	922	17	11	960	1150	39	38	1270	1670	63	100
			769	816	7	−19	768	844	10	14	755	908	23	10	876	1150	43	38
Plant	0·01	168	157	167	9	−0·5	158	174	15	5	178	214	37	29	227	290	66	75
			162	172	9	2	166	183	12	10	161	193	30	16	216	284	58	71
Control	0·05	843	789	838	1	—	755	829	2	—	690	828	3	—	634	834	4	—
Control	0·01		158	168	1	—	151	166	2	—	138	166	3	—	127	167	4	—
Standard	0·05	843	786	843	0	—	767	843	0	—	704	843	0	—	643	843	0	—

tested. The results of a preliminary experiment are given in Table 4 and those of a more carefully controlled experiment in Table 5.

The significance of this observation is that the roots of plants in solutions of the anhydride are exposed to progressively increasing concentrations of the insecticide as the solution is absorbed. It is important to remember that by the time about 80% of the solution has been absorbed the remaining solution may be about four times as concentrated as it was originally. To obviate this effect the solutions would have to be renewed or diluted daily.

Absorption by the roots from sand and soil

In previous experiments it was found that the aphids on plants growing in sand were killed by the anhydride sooner than those on plants in soil (David & Gardiner, 1951). It seemed probable that this difference occurred because the anhydride was absorbed more quickly from sand than from soil. This suggestion was confirmed by a preliminary experiment in which plants growing in sand and soil in $3\frac{1}{2}$ in. diameter pots were watered with 20 c.c. of an 0·1% solution of the radio-anhydride. When the plants were assayed after 5 days the radioactivity of those grown in sand was considerably higher than that of those grown in soil.

TABLE 6. *Radioactivity on the fifth day of plants grown in sand and soil treated with the anhydride*

	Part of plant		
Plant from	Upper half of stem (counts/g.)	Lower half of stem (counts/g.)	Roots and bean (counts/g.)
Sand	54	44	87
Soil	15	20	24

The same procedure was followed with an 0·05% solution of a more active sample of the anhydride to survey the distribution of radioactive material in the plants in greater detail. In this experiment each leaf on the plant numbering from the bottom was assayed separately. At the beginning of the experiment there were only four leaf pairs on the plants, but by the thirteenth day there were six pairs besides the crown of unexpanded leaves and the growing point. It can be seen from Table 7 that the level of radioactivity increases throughout the experiment, except for the tailing off observed on the thirteenth day with plants growing in soil. On the thirteenth day the maximum level of activity reached by the leaves of plants growing in sand and soil is equivalent to about 660 and 80 mg. of anhydride per kg. of plant tissue respectively. However, these quantities will not in fact occur, as it will be shown later that about half the anhydride absorbed is decomposed within the plant after 8 days. The observations made on two plants on the thirteenth day after treatment are summarized in Table 8. It will be noted that the lowest (oldest) leaves on

the plants are most damaged, although they do not contain a higher concentration of anhydride.

Table 7. *27 February 1950. Distribution of radioactive material in plants growing in sand and soil*

Day assayed	Counts/g./min. with leaf 1	2	3	4	5	6	Crown
			Sand				
1	9	—	—	15	-	—	-
2	46	66	58	17	-	—	-
4	257	334	322	384	172	—	—
9	560	540	810	718	362	135	—
13	1015	1115	1395	1215	1328	1016	458
			Soil				
1	2·5	—	—	1·0	-	—	—
2	12	10	21	11	-	—	—
4	41	40	55	52	18	—	—
9	100	161	184	173	113	50	—
13	119	140	164	150	113	109	56

Assuming no decomposition 100 counts/g./min. = 50 mg. anhydride per kg. of plant tissue. The activity observed was corrected for decay to day 1.

Table 8. *The condition of the leaves on plants on the thirteenth day after being treated with the anhydride*

	Plant in sand			Plant in soil		
Leaf	Condition	Counts/g. min.	Aphids	Condition	Counts/g. min.	Aphids
1	Marginal scorch, wilting and yellowing	1015	—	Slight marginal scorch	119	—
2	Marginal scorch, wilting and yellowing	1115	—	Normal	140	—
3	Marginal scorch, wilting and yellowing	1395	—	Normal	164	—
4	Marginal scorch, wilting and yellowing	1215	—	Normal	150	+
5	Slight marginal scorch and wilt	1328	—	Normal	113	—
6	Slight marginal scorch	1016	—	Normal	109	+
Crown	Trace of marginal scorch only	458	—	Normal	55	—

Persistence of the anhydride within the plant

The radio-anhydride may be decomposed in the soil before it is absorbed by the plant into radioactive but insecticidally inactive compounds. Likewise, once it is

in the plant it may be decomposed with the same result. Estimating the undecomposed anhydride involves separating it from the total aqueous extract obtained from the plant and assaying the separated material. This may be accomplished by the method suggested by Hartley & Heath (1950), already mentioned.

One ml. lots of an 0·1 % (v/v) solution of the anhydride containing radio-anhydride were fed to the plants by the cut tap-root technique (David & Gardiner, 1951). This method has the advantage that possible decomposition in the soil is avoided. Furthermore, the dose is taken up over a short period of time so that the rate of decomposition can be measured without the situation being complicated by the continual absorption of anhydride which occurs if the plants are grown in treated soil. The results of experiments of this kind are shown in Table 9. The fourth column of this table shows the total activity in the plants, the fifth the activity due to the undecomposed anhydride and the sixth this latter figure as a percentage of the total activity. It will be seen that about 50% of the anhydride is decomposed in 8 days. In column 7 the anhydride content has been calculated in terms of mg./kg. of plant tissue and in column 8 these values have been corrected for the fact that only about 70% of the radio-anhydride seems to be extracted from the plants (see Table 2). The 5- and 8-day figures have been corrected for decay of radioactivity to bring them in line with day 1. From column 4 it appears that on an average not more than about 10% of the anhydride and its decomposition products are lost from the plant in 8 days under the conditions of the experiment.

TABLE 9. *Decomposition of the anhydride within the plant*

Plant nos.	Days after treatment	Weight of plant (g.)	Total activity of plant tissue (counts/min./g.)	Undecomposed anhydride in plant tissue			
				Counts/ min./g.	%	Mg./kg.	Mg./kg. (corr.)
1	1	7·65	177	140	79	70	100
2		8·65	165	130	79	65	93
3	5	7·45	146	86	59	43	61
4		7·67	168	101	60	51	72
5	8	7·35	143	65	45	33	47
6		6·95	160	64	40	32	46

The dose of anhydride given in this experiment is about twice that known to be necessary to kill the aphids (David & Gardiner, 1951). It therefore appears that the anhydride is lethal to *Aphis fabae*, Scop. on broad beans when the concentration of undecomposed anhydride is about 50 mg./kg. of beans.

Transpiration of radioactive material

It has been concluded from the results given in Table 9 that comparatively little of the radio-anhydride, or its decomposition products which contain phosphorus, are lost from treated broad beans within 8 days. This conclusion is supported by the

fact that the solution obtained by condensing the material transpired by plants which have been absorbing an active 0·1% (v/v) solution of the anhydride for several days is not measurably radioactive.

Penetration and translocation following application of the anhydride to the leaves

It has been reported that the anhydride is absorbed by the leaves of certain plants and translocated in insecticidal quantities to other parts of the plant (Ripper, Greenslade & Lickerish, 1949; Ripper *et al.* 1950), but that this effect is difficult to demonstrate on the broad bean (David & Kilby, 1949; David & Gardiner, 1951).

Penetration

Most of the experiments to be described have involved translocation from the treated leaf into other parts of the plant. In one experiment, however, the distribution of the anhydride between the surface and the interior of treated leaves was investigated. A batch of similar bean plants was selected and the leaves were dipped in an 0·1% (v/v) solution of the anhydride containing 0·1% (v/v) 'Teepol' as a wetting agent. At various times after the leaves were dry seven pairs were cut from the plants. They were washed separately in two lots of 1·0% 'Teepol' using a small water-colour brush to scrub both surfaces. The leaves were then drained into the second wash, rinsed under running water, and boiled with 8% sodium hydroxide. The material washed off the leaves by the 1·0% 'Teepol' was assumed to be on the surface while that which could only be brought into solution by extracting the leaves with sodium hydroxide was considered to have penetrated into the leaves. Since the leaves varied in size the total amounts recovered on different occasions are not strictly comparable, but Table 10 shows that the percentage of total material recovered which can be washed off decreases with time, while that found in the extract increases. As would be expected, the penetration is at first rapid and later proceeds more slowly as the quantity of material on the surface of the leaves falls. It will be noted also that there is a steady decline in the amount recovered. As previous experiments suggest that very little material is lost by vaporization this loss can probably be accounted for by translocation to untreated parts of the plant.

Table 10. *Distribution of radioactive material between surface and interior of bean leaves after dipping in a solution of the anhydride, and its persistence in the dipped leaves*

Time after dipping (hr.)	Washed off		Extracted		Total counts	Percentage of total lost
	Counts/min.	Percentage total	Counts/min.	Percentage total		
1	43	93	3	7	46	0
5	28	62	17	38	45	2
24	11·9	30·5	27·2	69·5	39·1	15
72	6·1	19·5	24·7	80·5	30·8	33

Translocation

It is difficult to demonstrate that insecticidal quantities of the anhydride are translocated in the broad bean from treated to untreated leaves. By employing the radio-anhydride it can be shown that translocation does occur but that insecticidal concentrations are not usually built up in the untreated leaves. In other plants more material is translocated as is indicated by the death of the aphids and the level of radioactivity in the untreated parts.

Broad bean. To create conditions favourable for demonstrating translocation all the lower leaves of broad bean plants were dipped in 0·1% solutions of the radio-anhydride containing 0·1% 'Teepol' as a wetter, leaving only the undeveloped leaf crown and one expanded leaf pair undipped. The plants were dipped two or three times and assayed at various intervals. Table 11 provides definite evidence that translocation occurs under these conditions, but the untreated parts contained sub-lethal concentrations of the anhydride. The figures in the table are not corrected for decomposition of the anhydride and represent total quantity of active material present.

TABLE 11. *Translocation of radioactive material upwards from treated older to untreated younger leaves in the broad bean*

Plant nos.	Duration of experiment (days)	No. of treatments	No. of leaf-pairs dipped	Radioactive material (mg./kg.)	
				Treated leaf	Untreated leaf
1, 2	2	2	5	174	3·5
3, 4	5	3	4	242	5·9
5, 6	8	3	6	382	28·4

The aphids on the undipped parts of the broad beans used in the experiments reported in Table 11 were not killed. It was, however, noticed that the fully expanded leaves of plants 5 and 6 were much more heavily infested than the young leaves at the stem apex. An assay of these two parts separately showed that whereas the mature leaves contained only the equivalent of 18·9 mg./kg. radioactive material the stem apex contained 37·8 mg./kg.

Following this observation the parts of a plant which had been dipped three times starting 15 days previously were assayed. On this plant the two lowest leaf pairs

TABLE 12. *Distribution of radioactive material in broad bean plants following applications made to the lower leaves*

Part of plant assayed	Radioactive material (mg./kg.)
Bottom two dipped leaves	350
3rd leaf—undipped	11·0
4th leaf—undipped	16·5
5th leaf—undipped	20·3
Apex—undipped	28·3

had been dipped; at the beginning of the experiment there was one undipped fairly mature leaf pair and the apex. The results obtained are shown in Table 12. All the undipped parts assayed were infested with aphids. It may be concluded that the radioactive material passes preferentially into the actively growing parts of the bean.

Essentially similar results to those reported were obtained in experiments in which the plants were given a single treatment, but in this case the activity counts on the untreated leaves were very much lower.

Downward translocation of radioactive material from treated young leaves to untreated older leaves was also looked for. This occurs after repeated treatments of the young leaves but it is more difficult to demonstrate than the translocation in the opposite direction. The experimental plants had four fully developed leaf pairs. Two of these were treated with the same mixture of radio-anhydride and 'Teepol' (0·1% of each) as before, with the results shown in Table 13. Although the concentration of radioactive material built up on the treated leaves was very high very little material was translocated to the lower leaves. In a further experiment upward and downward translocation was compared in the same plants. The plants used had three pairs of mature leaves and the undeveloped crown. The two upper leaves were treated three or four times daily and the lowest leaf was left untreated. Five or six days later the untreated lowest leaf, the treated leaves and the new growth formed were assayed. From the results given in Table 14 it can be seen that much more radioactive material is translocated to the new growth than to the old leaves. In sampling the plant for upward translocation it is important to take only the new growth, as most of the radioactive material passes into this (p. 517).

Table 13. *Translocation of radioactive material downwards from treated younger to untreated older leaves of broad beans*

Plant nos.	Duration of experiment (days)	No. of treatments	Radioactive material (mg./kg.)	
			Treated leaf	Untreated leaf
1	4	13	430	8·4
2	4	13	618	2·6

Table 14. *Translocation of radioactive material downwards to older leaves and upwards to younger leaves from treated leaves on the middle part of the stems of broad bean plants*

Plant nos.	Duration of experiment (days)	No. of treatments	Below		Treated		Above	
			Wt. of leaves taken (g.)	Radio-active material (mg./kg.)	Wt. of leaves taken (g.)	Radio-active material (mg./kg.)	Wt. of leaves taken (g.)	Radio-active material (mg./kg.)
1, 2	5	12	2·40	4·5	2·80	812	2·15	71
3, 4	6	16	3·40	6·9	3·45	1120	2·30	43

Cabbage. Young cabbage plants were used. All except the growing point and one leaf of each plant was treated by dipping the leaves on the first 2–4 days of the experiment in the usual mixture of the radio-anhydride and 'Teepol' (each 0·1 %), and then drying the plants in such a way that none of the solution could come into contact with the untreated parts of the plant or run down the stem to the roots. Evidence of the translocation of radioactive material was found as shown in Table 15, but neither species of aphid was affected.

TABLE 15. *Translocation of radioactive material from treated older to untreated younger leaves in young cabbages*

Plant nos.	Duration of experiment (days)	No. of treatments	No. of leaves dipped total	Radioactive material (mg./kg.)	
				Treated leaf	Untreated leaf
1, 2	4	2	10	297	18·3
3, 4	7	3	10	384	21·5
5	14	3	5	310	11·5
6	14	4	6	364	17·6

Hop. Newly sprouted hop-sets with young shoots about 1½–3 ft. long were used. The usual radioactive solution was brushed on to some of the leaves and translocation to untreated parts was looked for. It will be noted that much more anhydride is held by the rough hop leaves than the smooth bean or cabbage leaves. This probably accounts for the high activities observed on the untreated leaves, which indicate a content of anhydride likely to be lethal to aphids. It would not be economically practicable to give commercially grown hops as many applications as employed in this experiment but, on the other hand, there would be relatively more old leaves on the plant at the time of spraying and these would provide a larger reservoir of anhydride to protect the new growth. Field tests show that new growth on sprayed hops is protected (Ripper *et al.* 1950).

TABLE 16. *Translocation of radioactive material from treated older leaves to untreated younger leaves and stem of hops*

Plant no.	Variety	Duration of experiment (days)	No. of treatments	Radioactive material (mg./kg.)	
				Treated leaf	Untreated leaf and stem
1	Eastwell Golding	3	7	1200	50
2	Bramling	5	14	1070	80
3	Eastwell Golding	5	14	812	132

Downward as well as upward translocation was looked for in a further experiment with hop-sets. The translocation was allowed to occur over 4–7 days during which

time eight to fifteen applications of the radio-anhydride were made. The results are given in Table 17*a*, *b*. It is evident that in the shoots of actively growing hop-sets some radioactive material is translocated downwards from treated leaves on the middle part of the stem to the older leaves below, but that larger quantities are translocated to the younger leaves. A few *Aphis fabae* Scop. established on the young growing tip of the Eastwell Golding hop in Exp. 17*a* were killed, as were strawberry aphids, *Pentatrichopus fragaefolii* Cock., on the untreated younger leaves of the hops used in Exp. 17*b*.

Table 17*a*, *b*. *Translocation of radioactive material upwards and downwards from treated leaves in the hop*

Plant nos.	Variety	Duration of experiment (days)	No. of treatments	No. of leaves and content of radioactive material: Treated leaves No.	Treated leaves Mg./kg.	Untreated older leaves below No.	Untreated older leaves below Mg./kg.	Untreated younger leaves above No.	Untreated younger leaves above Mg./kg.
				(*a*)					
1	Bramling	4	13	—	1245	—	3·0	—	50
2	Eastwell Golding	4	13	—	1960	—	4·5	—	477
				(*b*)					
1	Fuggles	4	8	6	507	7	16·4	6	29·6
2	Eastwell Golding	5	12	7	706	5	6·4	4	59·4
3, 4	OR55	7	15	14	1380	14	38·7	6	99·5

Pea. The lower leaves of young pea plants with vines about 6–8 in. long were dipped in the usual mixture of radio-anhydride and 'Teepol' (each 0·1%). About four compound leaves were treated but as they were injured by the solution, dipping was discontinued after three treatments. By the ninth day the treated leaves were badly withered and this naturally influenced the calculation of the quantity of radio-anhydride on these leaves on this date. Table 18 shows that radioactive material was translocated to untreated parts of the plants.

Table 18. *Translocation of radioactive material from treated older to untreated younger leaves in young pea plants*

Plant nos.	Duration of experiment (days)	No. of treatments	Radioactive material (mg./kg.): Treated leaf	Untreated leaf
1, 2	2	2	520	12
3, 4	4	3	805	40
5, 6	9	3	1455	147

Strawberry. It has been reported that aphids infesting the runners of strawberry plants may be killed by applying the anhydride to the parent plant only (Dicker,

1949). This interesting example of translocation has been confirmed using the radioactive anhydride 0·1% (v/v) containing 'Teepol' 0·1% (v/v). The plants used had overwintered outside but were potted and brought into the greenhouse with runners attached, about the middle of February, and 2 or 3 weeks later when new growth had formed they were used for the experiments. Some of the runners were allowed to root in separate pots but the others were unrooted and entirely dependent on the parent plant for water and nutrients. It is evident from the results given in Table 19 that, as would be expected, there is much more translocation from the parent plant to the unrooted runners. In these experiments the concentration of anhydride built up in the runner was insufficient to kill aphids present at the time of sampling. Probably this was because there were few leaves on the parent plants and not all of these were treated.

TABLE 19. *Translocation of radioactive material in the strawberry*

			Radioactive material (mg./kg.)			
			Main plant		Runners	
Plant nos.	Duration of experiment (days)	No. of treatments	Treated	Untreated	Rooted	Unrooted
1	2	4	294	2·5	0·2	3·0
2	5	10	725	26·7	—	4·2
3	4	9	—	—	—	4·5
	6	15	677	116	0·0	18·1

Detection of radioactive material in aphids and honey dew

By assaying aphids killed by feeding on plants treated with the radio-anhydride and the honey-dew they produced, it was shown that both contained radioactive material. Glass slides were arranged vertically near a broad bean stem heavily infested with aphids. The honey dew which they produced was projected out against the slides, while the aphids killed by the insecticide fell on to a filter-paper collar arranged further down the stem. In this way the falling aphids did not contaminate the honey-dew. The results of one such preliminary experiment are shown in Table 20.

TABLE 20. *Detection of radioactive material in aphids and honey dew*

Subject assayed	Weight (g.)	Total counts per min.	Radioactive material (mg./kg.)
Aphids	0·646	24·3	25·0
Honey dew	—	4·4	—
Plants from which aphids taken	6·2	3630	386

In a subsequent experiment six bean plants, heavily infested with aphids, were treated with three different concentrations of the radio-anhydride (1 c.c.) by the cut tap-root technique. A filter-paper collar was arranged around each plant to collect the aphids as they fell off. Five hours later about 50% of the aphids had fallen off

the two plants treated with the highest concentration. The remaining aphids were brushed off the plants and the whole batch from the two plants was weighed and assayed after heating for 20 min. with 2 N-sodium hydroxide solution, under a reflux condenser. Some time later when about 50% of the aphids had fallen from the plants, treated with the more dilute solutions, the insects were collected and assayed in the same way.

Table 21. *Concentration of radioactive material in aphids falling off treated plants*

Plant nos.	Conc. of soln. (%)	Calc. conc. of anhydride in plants (mg./kg.)	Radioactive material (mg./kg.)	
			Plants	Aphids
1, 2	0·20	153	119	36·5
3, 4	0·10	75·6	55·2	16·7
5, 6	0·05	34·1	22·9	14·2

The lowest concentration of anhydride in plants previously shown to free them entirely from aphids was 50 mg./kg. but 20 mg./kg. killed some aphids (David & Gardiner, 1951). It can be seen from the present experiments that when about 50% of the insects have fallen off plants containing about 23–55 mg./kg. anhydride (34–75% calculated) the whole population, on and off the plant, contains 14·2–16·7 mg./kg. anhydride. As the latter two figures are approximately equal although one lot of plants from which the aphids fell contained twice as much as the other, it appears that, correcting for extraction losses, about 15–20 mg. of anhydride per kg. of aphids is the 50% lethal dose. When the concentration of anhydride in the plant is increased further at the time 50% of the insects have fallen off, the population as a whole has taken more anhydride than is required to give a 50% kill.

Discussion

It has previously been shown that the anhydride is taken up by the intact roots of the broad bean (David & Gardiner, 1951). This is important because most of the plants used in the present experiments had some broken or rotten root tips, and it might be thought that the limited absorption of anhydride observed to occur might have taken place through these damaged parts. The fact that selective rejection of the anhydride occurred suggests that these damaged roots soon ceased to function or contributed but a small part of the material used by the plant.

Fertilizer phosphate added to soil even in dilute solution is known to be held by the upper layers (Russell, Adams & Martin, 1949). This observation may explain why the anhydride is more readily absorbed from sand than from soil. The slower absorption from soil could occur because the material is held in the top layer of the soil, but not in the sand, and therefore only reaches some of the roots; while even if it does become evenly distributed in the small pots used in these experiments it may be much more strongly absorbed by soil than by sand. If the anhydride is

strongly absorbed by soil it would be inefficient as a systemic insecticide intended for absorption by the roots of plants except where there is an extensive root system near the surface of the soil. It is likely too that some soils, according to their composition, would be more absorptive than others.

In tests where aphid-infested leaves are dipped in solutions of the anhydride containing a wetter it has been assumed that the material is acting as a contact poison, though it was recognized that it might enter the leaf and act as a stomach poison. Experiments on the penetration of the anhydride into leaves show that this occurs relatively rapidly (Table 10), and careful timing and examination of the aphids will be necessary to decide between the two explanations. In addition, it may be possible to treat aphids off the leaves, but in preliminary tests there was a high mortality of adult apterae dipped in the wetting agent alone.

The radioactive technique provides convincing confirmation that radioactive material can be translocated from treated to untreated leaves. And coupled with the insecticidal effect observed there seems little doubt that some of the radioactivity comes from unchanged anhydride which in other circumstances has been isolated from plant tissue. The experiments show that much more material is translocated to young growing leaves than to leaves older than those treated, although downward translocation also occurs. It is evident that in general the radioactive material is distributed mainly in the direction in which the plant is moving other materials. This movement has been shown to occur to the growing point but may also occur in the direction of the roots when a perennial plant is building up reserves or preparing to overwinter.

An estimate has been made of the median lethal dose of the anhydride to *Aphis fabae*. Without taking special measures this value is not easy to determine accurately. The first difficulty is to obtain an adequate number of insects of the same instar established on a suitable plant. No attempt was made to do this, a mixed population being used. Another difficulty is that while most insects fall off the treated plant while still capable of moving, others die clinging to the plant. Further, they respond after quite widely differing times so that those affected first begin to dry and lose weight while the others are still on the plant. In order to get the aphids off the plants over as short a time as possible it is necessary to dose the plants fairly heavily and collect the aphids as soon as about 50% have fallen off.

I should like to thank Dr D. N. McArthur and Dr J. G. Hunter of the Macaulay Institute for Soil Research for agreeing to lend the Geiger counting equipment used in this investigation and Dr R. A. E. Galley for arranging the loan, Dr B. A. Kilby and Mr J. E. Gardiner who kindly made and supplied the radio-anhydride, and Mr B. O. C. Gardiner who assisted in the experiments.

REFERENCES

Bennett, S. H. & Martin, H. (1947). The qualitative examination of insecticidal properties. Progress report 1947. *Ann. Rep. Long Ashton Res. Sta., Bristol*, p. 147.

Bennett, S. H., Martin, H., Stringer, A. & Woodcock, D. (1948). The qualitative examination of insecticidal properties. Progress report 1948. *Ann. Rep. Long Ashton Res. Sta., Bristol*, p. 138.

David, W. A. L. (1950). Insecticidal action of radioactive bis (bis-dimethylamino)-phosphonous anhydride. *Nature, Lond.*, **166**, 72.

David, W. A. L. & Gardiner, B. O. C. (1951). Investigations on the systemic insecticidal action of sodium fluoroacetate and of three phosphorus compounds on *Aphis fabae* Scop. *Ann. appl. Biol.* **38**, 91.

David, W. A. L. & Kilby, B. A. (1949). Preparation and insecticidal action of bis (bis-dimethylamino)-phosphonous anhydride. *Nature, Lond.*, **164**, 522.

Dicker, G. H. L. (1949). The strawberry aphid *Pentatrichopus fragaefolii* (Cock.), and its control. *Ann. Rep. E. Malling Res. Sta.* p. 132.

Gardiner, J. E. & Kilby, B. A. (1949). A radioactive systemic phosphorus insecticide. *Research*, **2**, 590.

Gardiner, J. E. & Kilby, B. A. (1950). Organic phosphorus insecticides. Pt. 1. Synthesis of bisdimethylaminophosphonous anhydride containing ^{32}P. *J. chem. Soc.* p. 1769.

Hartley, G. S. & Heath, D. F. (1950). Radioactive Pestox 3. (Private communication from Messrs Pest Control Ltd., Cambridge.)

Ripper, W. E., Greenslade, R. M. & Hartley, G. S. (1950). A new systemic insecticide bis (bis-dimethylamino-phosphonous) anhydride. *Bull. ent. Res.* **40**, 481.

Ripper, W. E., Greenslade, R. M. & Lickerish, L. A. (1949). Combined chemical and biological control of insects by means of a systemic insecticide. *Nature, Lond.*, **163**, 787.

Russell, R. S., Adams, S. N. & Martin, R. P. (1949). Radiation effects due to phosphorus-32 in fertilizer experiments. *Nature, Lond.*, **164**, 993.

Schrader, G. (1947). The development of new insecticides. *B.I.O.S. Final Report*, no. 714 (revised) 63 pp. London: H.M.S.O.

Veall, N. (1948). A Geiger-Müller counter for measuring the beta-ray activity in liquids, and its application to medical tracer experiments. *Brit. J. Radiol.* **21**, 347.

(*Received* 16 *August* 1950)

PROCEEDINGS OF THE ASSOCIATION OF APPLIED BIOLOGISTS

Ordinary Meeting of the Association held on Friday, 29 September 1950, in the Imperial College of Science and Technology, London; the President, Mr G. Fox Wilson, in the Chair.

The following papers were read and discussed:

1. Entomological research in the Overseas Food Corporation (Tanganyika). By Dr A. C. Evans.
2. Mushroom nutrition and fructification. By Dr R. L. Edwards.
3. Bypaths in mushroom research. By Dr R. L. Edwards and Dr S. Burrows.

Joint Meeting of the Association with the British Mycological Society held on Friday, 20 October 1950, in the Imperial College of Science and Technology, London; chairman at the morning session, the President, Mr G. Fox Wilson, and at the afternoon session the President of the British Mycological Society, Dr G. C. Ainsworth.

The plant and the law

The following papers were read and discussed:

1. Legislation against plant diseases and pests in England and Wales. By Mr W. C. Moore.
2. Some repercussions arising from the exchange of plant material between countries. By Dr S. P. Wiltshire.
3. Inspection procedure in England and Wales. By Mr A. P. Winsor.
4. The Sugar Beet Eelworm Order 1943. By Dr F. G. W. Jones.
5. The Progressive Wilt of Hops Order. By Dr W. G. Keyworth.
6. Some scientific aspects of the certification schemes for fruit plants and hops. By Dr R. V. Harris.

Ordinary Meeting of the Association held on Wednesday, 8 November 1950, in the Imperial College of Science and Technology, London; the President, Mr G. Fox Wilson, in the Chair.

The following papers were read and discussed:

1. Marking and breeding *Testacella* slugs. By Dr H. F. Barnes and Miss B. M. Stokes.
2. Technical and biological aspects of insect drift. By Dr C. G. Johnson.
3. Coloration of Colorado beetle wings. By Mr E. W. Dunn.
4. The relative efficiency of some insect traps. By Dr C. B. Williams.
5. The preferences shown by honeybees for certain nectars. By Miss G. Wykes.
6. The expression of symptoms of leaf-roll virus in potatoes. By Mr J. H. Wilson.
7. The effect of microclimate on the establishment of timothy (*Phleum pratense*). By Mrs S. S. Williams.

ENTOMOLOGICAL RESEARCH IN THE OVERSEAS FOOD CORPORATION (TANGANYIKA)

By A. C. Evans, *Overseas Food Corporation*

A general description of the three areas being developed by the Corporation has been given by Bunting (1950).

For the first 2 years entomological work had to be carried out single-handed by the writer except for the able assistance of the regional plant pathologist at Urambo in the second year, for studies on rosette disease. Two more entomologists were appointed in time for the beginning of the third year, and next year will see a plant pathologist and an entomologist in each of the three regions for the first time.

Rosette disease

This virus disease with an aphid vector is a serious threat to groundnut cultivation in all parts of Africa. Research is being conducted along three main lines: epidemiology in the field, the use of insecticides, and plant-vector studies. The last-named line is being followed by Dr H. H. Storey, of the East African Agricultural and Forestry Research Organization.

Rosette struck the groundnuts at Urambo severely during the first production year, 1948–9, almost all the plants on the production areas being infected to a greater or lesser extent, and it was estimated that 40% of the crop was lost as a result. Only small-scale agricultural operations have been carried out in the Southern Province, and so far rosette disease has not been serious there, but examination of groundnuts on native gardens suggests that the disease may prove serious in the not too far distant future. Only infrequently have isolated rosetted plants been found at Kongwa.

It was considered important to locate the source of infection at Urambo. The first obvious site was the surrounding bush, but intensive searching failed to discover the vector, and transects across the production areas did not show a fall in the gradient of infection from the bush. It is now considered that the infected vectors were blown in on the prevailing easterly winds from the large areas of native cultivation which begin about 13 miles to the east of our area and extend to and around Tabora, 45 miles farther east. The aphids probably originated from colonies which had survived the dry season on volunteer groundnut plants. Alternate hosts which could be considered to be of economic importance have not yet been discovered. The gap between seasons is bridged on volunteers which grow from unharvested nuts. The extent to which volunteers can occur is shown by the following analysis of fifty-seven plots, each one-fortieth of an acre:

	No. of plots
No volunteers	4
Healthy plants, no aphids found	21
Healthy plants, some aphids found	13
Some rosetted plants, no aphids found	8
Some rosetted plants, some aphids found	13

There was an average of 1300 plants per acre and more than half the plots presented a serious threat to the next crop. The number of volunteers can be considerably reduced by improved harvesting methods, but it will still call for a high degree of skill on the part of the African tractor driver to exterminate volunteers during seed-bed preparation.

Early planting and close-spacing are considered to be practical measures to combat rosette disease. Data for one year only (1948–9) are available at Urambo, since extremely few infected plants occurred in 1949–50. The value of early planting is clearly shown in a date of planting experiment. The first planting was 3 weeks late for the season, owing to unavoidable delay in preparing a new farm area for its first season.

TABLE 1. *Effect of planting date on incidence of rosette disease in groundnuts, Urambo, 1948–9*

Date of planting	Plant numbers (thousands/acre) Total at harvest	Rosetted plants at 15 Mar.	Yield groundnuts (lb./acre)
18 Dec.	51·0	3·5	989
27 Dec.	46·8	5·9	814
2 Jan.	33·3	13·5	550
10 Jan.	37·4	16·0	361
19 Jan.	36·2	20·1	86
29 Jan.	41·5	14·9	86

It is quite clear that the two earliest plantings did not develop a heavy infection, but later plantings were progressively more heavily attacked. Plantings later than 29 January were so heavily attacked that by harvest infection counts showed 100% infection and no yield was obtained.

The effect of close spacing was studied in a factorial experiment with three spacings in the row, and three spacings between rows with three dates of planting of three varieties.

TABLE 2. *Effect of spacing on the incidence of rosette disease in groundnuts, Urambo, 1948–9*

	Plant numbers (thousands/acre) Spacing within rows			Significant differences	
	3¼ in.	6½ in.	9¾ in.	5%	1%
Total plants	50·8	35·9	26·7	4·9	6·1
Rosetted plants	16·8	17·2	17·5	3·0	3·8
	Spacing between rows				
	18 in. (S)	28 in. (S)	36 in. (D)		
Total plants	44·2	27·9	41·2	4·9	6·1
Rosetted plants	18·6	15·5	17·3	3·0	3·8

(S) single rows; (D) double rows, 6 in. apart, with 36 in. between centres of double rows.

In spite of wide variations in the populations achieved, no reduction in the incidence of disease was achieved by establishing higher populations of total plants.

If the appreciation of rosette disease given above is correct, namely that the disease is brought into our areas by air-borne vectors, that the early plantings are more resistant and that close spacing is not a control measure, then it is necessary to look into the possibilities of insecticidal control for further extending the planting season. The number of applications of dusts or sprays based on DDT or similar materials would be prohibitive on account of expense, but the recently developed systemic insecticides show more likelihood of promise. Seven phosphorus-containing insecticides were compared and the most successful, based on bis (bisdimethylamino) phosphonous anhydride, protected plants for 24 days from infestation by aphids applied every fifth day. Even after 40 days the insecticide was exerting some effect. The insecticides were applied at the rate of 0·5 ml. of a 1% solution of the maker's concentrate.

The results could not be followed up in the field in the following year (1949–50) as the disease was scarcely present.

Other pests of groundnuts

Groundnuts have been remarkably free from other pests. Army worm, *Laphygma exempta*, occurred in numbers on restricted small areas at Kongwa, but were consumed by migrating flocks of Abdim's storks. Nymphs of grasshoppers were found feeding on the leaves, but the

damage was negligible. *Hilda patruelis* Stahl., which has caused serious losses to groundnuts in parts of Tanganyika, has been found on composite weeds at Urambo and Kongwa, but rarely on crops.

Termites

Termites have caused considerable losses in sunflowers in all three regions and appreciable losses in maize. The earlier ripened plants which have to stand until the crop is mechanically harvested are particularly susceptible. They are felled at ground level by the termites and are a total loss unless gleaning is possible. In the Southern Province termites caused considerable damage in one year by felling green plants. Groundnuts are particularly liable to damage after digging when the crop is lying on the ground waiting for the combine harvester. This type of damage can be prevented by careful timing of harvesting operations. Damage to standing crop is far more difficult to prevent. Even-ripening strains of seed and planting at uniform depth will reduce the loss to some extent, and continued cultivation of the ground may also reduce the termite population in time. The application of suitable insecticides with the fertilizer offers one possible line of attack. DDT and BHC were applied with the fertilizer in a sunflower experiment in Southern Tanganyika in 1949–50. DDT had no effect, but BHC reduced the loss from 66 to 33%. In this case the plants were allowed to stand longer than usual in the field. The chemical control of termites may be a dangerous weapon until the role of termites in agricultural land is known. Also BHC may be found to taint the oil of succeeding crops of groundnuts.

Calidea dregei

This pentatomid has caused much damage to sorghum and sunflower at Kongwa and Urambo. It attacks the grain of sorghum in the milky stage and leaves it shrivelled. The husk of sunflower seed is too hard for the bug to penetrate, but it pushes its proboscis through the micropyle and feeds on the kernel. Although the seeds are apparently undamaged, a considerable loss can be experienced. It has been found that the weight of kernel is reduced but the weight of husk is increased. The oil content in the kernel is slightly reduced, but its free fatty acid content can be high, in the neighbourhood of 10%. Germination of damaged seed is considerably decreased. Populations of *Calidea* of the order of 140,000 per acre have been recorded at Urambo. Heavy dusting with DDT, BHC and pyrethrum powders, and air spraying with 0·4% pyrethrins in diesel oil at 1 gal./acre, failed completely to exert any measurable control. Work with systemic insectides gave inconclusive results.

Miscellaneous

Stemborers, *Busseola fusca*, caused much damage to the combine types of sorghum grown on the experimental farm at Kongwa in 1947–8, nearly 100% of the stems containing larvae or pupae or pupal cases. The resultant weakening caused most of the stems to break as a result of the weight of the head, or through wind action. The effects of the combined attacks of *B. fusca* and *Calidea dregei* was not encouraging for large-scale plantings of sorghum, and in 1948–9 the crop was grown on the experimental farm only. A comprehensive programme was drawn up in that season to study the life history and control measures but very little damage was experienced. In 1949–50, large-scale production was introduced and damage was not severe.

Maize has only been attacked in restricted patches, but the pest is a severe potential pest to grain production in the Kongwa area.

Central-shoot fly, *Atherigonia indica infuscata* Emd., caused much damage to sorghum in 1948–9, by destroying the main shoots and primary tillers. The resultant crop was reduced in weight and ripening was spread over a long period by a continued production of small tillers.

About 15% of the maize cobs were damaged by cobworms in some areas at Kongwa in 1948–9, but the damage to individual cobs was slight.

Pollination studies on sunflowers

The sunflower strains available at the present time are insect pollinated. Bagged heads set 10–20% only of seed compared with 75–95% for unbagged heads. During the first year of extensive sunflower planting the percentage of unfertilized (light) seeds seemed to be high. It is generally considered that in a dry season the proportion of empty hulls is greater than in a normal season, and the season 1948–9 was a drought year. Extensive sampling and analysis of seed, however, showed that there was a mean of 14% unfertilized seed even from plants which grew where local moisture conditions were good, as in drainage hollows. The percentage of unfertilized seeds varied considerably from field to field (10–18%) and a highly significant negative regression was observed between percentage unfertilized seed and percentage undeveloped (blind) seed, this latter being considered a useful numerical expression of local drought conditions. It is thought that under drier conditions the secretion of nectar may be less than under moister conditions and so the bees might have to visit more florets to obtain full loads of nectar in the drier fields.

The numbers of bee-nests in various fields were estimated. An average of 6·25 per field were found in fields growing sunflower, 2·25 per field in fields under groundnuts for 2 years and 1·50 per field in recently cleared land. Not very large numbers per field (550 acres) when Russian workers consider one colony per 5 acres necessary for average yields. Most of these colonies were robbed before the end of the season by native honey hunters.

Uncleared bush might be thought to provide an inexhaustible supply of pollinators, but the percentage fertilization one-third of a mile from bush was found to be 6·5, at two-thirds of a mile away and farther it was found to be 14·5, a highly significant difference.

Thus it appeared that there was an insufficiency of insects to pollinate the crop. With further extensive planting of sunflowers in 1949–50 a close watch was kept on the pollination problem, but no improvement was noted. Indeed, the percentage of unfertilized seed rose to about 50.

REFERENCE

Bunting, A. H. (1950). Technical aspects of the East African groundnuts organization. *Ann. appl. Biol.* **37**, 699–704.

LEGISLATION AGAINST PLANT DISEASES AND PESTS IN ENGLAND AND WALES

By W. C. Moore

Ministry of Agriculture and Fisheries Plant Pathology Laboratory, Harpenden, Herts

Few people have more than the haziest idea of the plant legislation in force even in their own country or of the principles underlying it, and as my present object is merely to provide an intelligible introduction to the papers that follow, I shall do no more than attempt to summarize in simplified form the existing plant disease legislation in England and Wales and to indicate some of the reasons underlying governmental action. Those who want precise and detailed information about particular legislation must consult the appropriate Orders issued by the Ministry of Agriculture and Fisheries, and they should appreciate that the various Orders now in force are liable to be extended, revoked or revised at any time.

In general, plant disease legislation in this country has two aims, namely, to prevent the introduction and spread of NON-RESIDENT foreign diseases and pests, and to control the spread of important RESIDENT diseases and pests.

The first line of defence against the NON-RESIDENTS is provided by the *Importation of Plants Order of* 1947, which is designed to ensure, as far as possible, that only healthy plants or parts

of plants are imported. Certain plants are prohibited importation altogether from some or all countries. Thus, potatoes are prohibited entry from European France as a protection against Colorado beetle and from North America for the same reason, as well as to exclude the introduction of bacterial ring rot (*Corynebacterium sepedonicum*) and certain virus diseases, including spindle tuber and witches' brooms, not yet known in Britain. Recently, chestnut trees (*Castanea*) have been added to the prohibited list because of the devastating effect of the introduction of chestnut blight (*Endothia parasitica*) into Italy. These, together with poplar trees and certain pines, are dealt with under a special Order concerned with forest trees (*The Importation of Forest Trees* (*Prohibition*) *Order of* 1949), issued by the Ministry at the request of the Forestry Commission.

Apart from the prohibited plants, any plants or parts of plants intended for planting can freely enter the country provided they are accompanied by a Health Certificate issued by the Phytopathological Service in the country of origin. Seeds do not need a health certificate at all but with many plants and vegetables from European countries an additional certificate is required in order to guard against the introduction of Colorado beetle. The ordinary Health or Phytosanitary Certificate has its limitations of course: it is based on a pre-export examination when most plants are in a dormant condition, so that it cannot cover virus diseases, and no health certificate can be expected to guarantee absolute freedom from diseases and pests. Nevertheless, check inspections of imported consignments carried out over many years have shown that the purchaser is ensured a reasonably sound and healthy commodity. Difficulties are provided for, and overcome by, issuing special licences, which enable, for example, collectors to import wild alpine and other plants, and individuals or institutions to bring in otherwise prohibited material for scientific study.

The second line of defence against non-residents lies in the operation of the *Destructive Insects and Pests Order of* 1933 which, like all the other Orders aimed at plant parasites, is issued under the general umbrella of the *Destructive Insects and Pests Acts*, 1877 *to* 1927, originally passed into law in 1877 to deal with the threat of Colorado beetle. There are now special Orders (*Colorado Beetle Orders of* 1933 *and* 1950) to combat the Colorado beetle and to give effect to the Ministry's policy of complete extermination of this pest. The main D.I.P. Order of 1933 may be brought into operation as soon as unwanted parasites are found in the country. It permits steps to be taken to prevent the spread of any 'insect of a non-indigenous species' by which is meant any insect, fungus, bacterium or transmissible virus, not established in Great Britain before 1933, which is destructive to agricultural or horticultural crops or to trees or bushes. In practice this Order is usually of a 'come and go' nature. That is to say, action may be continued against a particular parasite for some years and then dropped, either because the parasite is exterminated, or because it spreads to such an extent that legislative action under the Order is no longer appropriate (as happened with chrysanthemum midge) or because it can be more suitably dealt with as an advisory problem (e.g. bacterial canker (*Corynebacterium michiganense*) of tomato).

Another provision of this Order, of much significance to research workers, is a prohibition on the keeping, sale or release of a non-indigenous insect (as defined above) except under licence. It is not generally realized to what extent this law governs international exchange of infected or infested plant material as well as fungus or bacterial cultures. Licences (to which certain conditions are attached) are freely issued to responsible applicants and in practice it is usually possible to frame the requirements so that national interests are safeguarded without undue interference with research.

Action against RESIDENT diseases and pests is again of a twofold nature, namely: to prevent further spread of certain parasites, and to prevent the sale of planting material obviously infected with certain diseases or pests. The *Sugar Beet Eelworm Order*, 1943, will be discussed by Mr Jones and the *Progressive Verticillium Wilt of Hops Order*, 1947 by Dr Keyworth. The last-named disease, wart disease of potatoes, and Colorado beetle are at present the only notifiable diseases and pests. *The Wart Disease of Potatoes Order of* 1941, among other things, compels the destruction of affected material, restricts potato planting on contaminated land

to approved immune varieties, and prohibits the planting and sale for planting of foreign potatoes except under licence. Special provisions are made for protecting the area around the Wash, which is particularly concerned with the export trade in seed potatoes. The results of research carried out many years ago by Prof. F. T. Brooks and his colleagues form the basis of the *Silver Leaf Order of* 1923 under which dead wood of apple and plum trees must be destroyed by fire before 15 July in each year.

Under the *Sale of Diseased Plants Orders of* 1927 *to* 1943 it is an offence to sell, offer or expose for sale, plants substantially affected with certain named pests and diseases (including club root, American gooseberry mildew, fruit tree cankers, woolly aphid, scale insects and rhododendron bug) or potato tubers or narcissus plants or bulbs which are *visibly* rendered unfit for planting by reason of attack by any disease or pest.

On the borderline of our subject are the Ministry's Health Certification Schemes for potatoes, strawberry, black currant, raspberry, fruit stocks and hops. These are designed primarily as an effective weapon against virus diseases by providing the grower with healthy planting stock of guaranteed purity. Some of them are purely voluntary schemes but three are tied to special Orders. Thus, under the *Sale of Strawberry Plants and Black Currant Bushes Order*, 1946, only certified strawberry runners and black currant bushes may be sold (except under licence). Similarly, under the *Wart Disease of Potatoes Order of* 1941 only certified potatoes may be planted though, owing to seed shortages and the need for the maximum potato acreage, it proved necessary to relax the restrictions temporarily, and each year since 1941 general licences have therefore been issued permitting the planting in most areas of any potatoes other than foreign ones.

I have dealt only with the plant legislation in England and Wales. Within the United Kingdom, Scotland and Northern Ireland have their own regulations and most other countries have introduced similar legislation of one kind or another. There is clearly a need for co-ordinated international action to simplify phytosanitary certificates, to secure uniformity in quarantine requirements, to ensure that plant quarantines are based only on phytosanitary grounds and on sound biological knowledge, and, for mutual advantage, to exchange information about dangerous diseases and pests. All this is envisaged in a new International Plant Protection Convention now under discussion and sponsored by F.A.O. It also provides a programme of work for the European Plant Protection Organization about to be established, and for other projected regional organizations with similar aims in Africa and south-east Asia.

INSPECTION PROCEDURE IN ENGLAND AND WALES

By A. P. Winsor

Ministry of Agriculture and Fisheries, 1–4 Cambridge Terrace, Regent's Park, London, N.W. 1

Most countries throughout the world have some form of phytopathological service. Some of these services are very much more intricate than others, and about 30 years ago there was a tendency for the world to be divided into two parts, the 'old' and the 'new'. Those countries forming the 'old' world had comparatively simple plant import regulations, whilst those forming the 'new' world had introduced complex regulations in an effort to prevent the introduction of pests and diseases which would endanger their crops of economic importance. The latter type of action is quite understandable when it is appreciated that vast tracts of virgin land were being brought under cultivation, and crops, which were of recent introduction and unaccompanied by many of their common parasites, were being grown. The position to-day has, however, been radically changed by the advent of faster travel, notably by aeroplane, thus increasing the speed at which plant life and insects may be conveyed from one part of the world to the other. This new factor, along with the discovery of diseases over which there is no known control, has caused many countries to revise their plant import regulations.

During the period between the two world wars, an excellent phytopathological service, the inspectorate side of which comprised very largely the Education Inspectorate of the Ministry of Agriculture and Fisheries, was built up in England and Wales. This staff numbered about thirty and its members were chosen for their aptitude for work of this type. After 1919 each officer recruited to the service was required to hold a degree in agriculture or horticulture or its equivalent (for example, the National Diploma in Agriculture or Horticulture), and to have had practical experience. The total number of officers employed on the Education Inspectorate was approximately fifty, comprising one chief inspector, one deputy chief inspector, seven divisional inspectors, thirteen general inspectors and thirty-two inspectors. The senior officers were engaged mostly on the administration of their divisions and work dealing with agricultural education (including horticulture).

Inspection work in the 'field' was almost entirely carried out by general inspectors and inspectors, officers possessing good observational powers and a distinct aptitude for close investigation and attention to detail. The administration of all Acts, Orders and Regulations, under which such work was undertaken, was the responsibility of the Ministry's Horticulture Branch, now known as Horticulture Branch I, and the scientific side of the work was assigned to the Plant Pathology Laboratory, Harpenden. Inspectors had ready access to the laboratory staff to whom they looked for advice on all matters of a scientific nature. The work proceeded very smoothly, there being an excellent team spirit.

In October 1946, when the National Agricultural Advisory Service (N.A.A.S.) was formed and the Education Inspectorate was abolished, the staff was absorbed by the new service, filling various posts at headquarters, provincial headquarters, and in the counties, thus causing the work previously performed by comparatively few to be distributed among a much larger staff, many members of which had to receive training in inspectorial duties. To meet this situation, training and refresher courses were arranged to coincide with those periods of the season when the crops and the pests and diseases to be identified were most easily or readily recognized. These inspectorial duties have been divided between agricultural and horticultural officers, the former undertaking work concerned with potato crop certification, together with work under the Sale of Diseased Plants Orders where potatoes are involved, and the latter undertaking work in connexion with all other crops coming within the scope of the plant certification schemes, Sale of Diseased Plants Orders, etc. The present arrangements are somewhat loosely knit, but every endeavour is being made to obtain a proper functioning.

At this stage I propose to divide my subject into three parts:

(*a*) The steps taken to meet the requirements laid down by other importing countries.

(*b*) What is done to prevent the introduction into the U.K. of dangerous pests and diseases.

(*c*) The measures taken to prevent the spread of pests and diseases and to raise the standard of health of material for planting.

(*a*) *Inspection of plants for export*

It is necessary for the nurseryman or exporting firm to send an application to the Ministry of Agriculture (Horticulture Branch I) giving details of the nature and number of plants and the country to which they are to be exported, together with the date of intended despatch. Instructions are then sent to the appropriate inspecting officer who arranges with the exporting firm to carry out the inspection. Owing to the difficulty of keeping inspecting officers completely up-to-date with the ever-changing regulations of importing countries, it is very desirable that this procedure be followed.

The exporter is required to have all plant material assembled ready for inspection at the time of the inspecting officer's visit. This requirement may embrace all the plants forming a consignment, or samples of them where the inspection concerns large numbers of plants of one type or variety, e.g. manetti rose stocks. Some countries will not allow soil (sand and earth) to accompany imported plants and it is part of the inspecting officer's duty to see that the plant roots are free from soil, sand or earth, and that the packing medium is clean and

free from soil, etc. In some instances a limited choice of packing materials is imposed by the importing country and the officer ascertains that an acceptable material is to be used. Some countries will allow the importation of plants, bulbs, etc., only under permit, a copy of such permit being sent to the exporter specifying the plants and the period for which the permit is valid.

Plants for export presented for inspection should be made ready by removal of dead or excess foliage and be quite free from any pest or disease. Usually with small consignments exporters are given, at the discretion of the inspecting officer, the opportunity of replacing any odd plants which may be rejected. It is important that certain plants should be prepared for export by reason of their ability to withstand transit conditions—some will travel best dry and others moist—and it is not adequately recognized how important a part correct packing will play in the arrival of the plants at their destination in a sound and live condition. The correct type of packing of plants, bulbs, etc., destined for all parts of the world can be made a very fascinating study.

Occasionally, where the regulations of the importing country demand it, the certificate of health is issued by the inspecting officer; otherwise all certificates are issued by Horticulture Branch I on the recommendation of the inspecting officer. The form of certificate in use to-day varies considerably; some fifty-three are in use at the present time to meet the requirements of importing countries. The restrictions on certain species of plants imported by some countries make it necessary that the plants be examined during the growing season, to ensure freedom from specific pests, diseases and viruses. This entails a summer inspection of many nurseries. More recent legislation introduced by countries in North America and North Africa make it necessary to certify that plants and potatoes have been grown in soil free from the potato root eelworm and in some instances bags containing potatoes for export must be officially sealed.

Little imagination is needed to appreciate the very large amount of work involved and the time spent in enabling plant (and potato) exporters to comply with the multifarious plant import regulations.

(*b*) *Importation of plants*

In common with many other countries, the United Kingdom also has laws governing the importation of living plants and certain raw fruits and vegetables for the purpose of preventing the introduction of destructive pests and diseases. Reference has already been made to the Destructive Insects and Pests Acts of England and Wales, under which all Orders are at present made for dealing with pests and diseases dangerous to agricultural and horticultural crops. Under the D.I.P. Order 1922, living plants or parts thereof arriving at our ports had to be accompanied by a certificate of health; if the plants arrived unaccompanied by this certificate they were allowed to proceed to their destination, but the Ministry of Agriculture was advised of their entry by H.M. Customs and Excise. The weakness of this procedure is illustrated by the fact that on many occasions a consignment (plants, potatoes, etc.) had been disposed of before an inspection could be arranged. In 1933, with the imposition of tariffs, the position was changed by the introduction of the Importation of Plants Order 1933. This Order provided for the prohibition of any plants, etc., arriving at our ports unaccompanied by the relevant certificates and the plants were detained at the port of landing pending authorization of entry and disposal after inspection. This was a very definite step forward in the efforts made to prevent the introduction of pests or diseases, and about this time the work of making check inspections at ports and warehouses was intensified, with the result that the Ministry was able to form an opinion on the validity of the certificates which accompanied imported plant material coming within the scope of the Order.

An importer of a consignment of plants detained at the port of entry because the phytosanitary certificates are not in order must apply to the Ministry for its release. This may be arranged following an inspection indicating that the plants are healthy, for which service a fee is payable. Should the consignment of plants be found unhealthy owing to the presence of

pest or disease, the inspecting officer serves a notice on the importer (or his agent) requiring him (*a*) to destroy all or part by fire forthwith, or (*b*) to re-export, or (*c*) to treat in a prescribed manner so as to render the pest or disease innocuous. Much is left to the discretion of the inspecting officer as to what directions he may give; but he has the advantage of consulting the Director of the Plant Pathology Laboratory whenever he is in doubt as to when any one or all of these measures are applicable. It is the responsibility of the inspecting officer to see that any measures imposed by him are completely carried out.

Needless to say, it is not possible to inspect every plant in a consignment, unless it happens to contain a small number. The inspection, therefore, must be on a sample basis, but an inspecting officer very quickly becomes expert at deciding which are the more important items in a consignment to receive his more detailed inspection; particularly is this so when carrying out check inspections. Such work is facilitated by the fact that importers must present packing lists to the Customs officer, to which document the inspecting officer may have access. From experience he becomes cognizant of those subjects likely to carry infestation or infection and where on the plant or bulb to look for symptoms or traces of these as they may not be readily detected. Here are a few examples:

Narcissus	Basal rot (*Fusarium bulbigenum*)
Narcissus	Smoulder (*Sclerotinia narcissicola*)
Tulips	Fire (*Botrytis tulipae*)
Colchicum	Rust (*Uromyces colchici*)
Gladiolus	Hard rot (*Septoria gladioli*)
Gladiolus	Dry rot (*Sclerotinia gladioli*)
Gladiolus	Scab (*Bacterium marginatum*)
Iris	Eelworm (*Ditylenchus destructor*)
Rose stocks	Rust (*Phragmiduim mucronatum*)
Cherries	Fruit fly (*Rhagoletis cerasi*)
Potatoes	Tuber moth (*Phthorimaea operculella*)

(*c*) *Internal regulations*

Having taken certain precautions for the prevention of the introduction of dangerous pests and diseases it would be illogical if we did not adopt some measures by which we might attempt to prevent the spread of the more harmful ones, in particular those over which there is no known control. Examples of these are Wart Disease of potatoes and Progressive Verticillium Wilt of hops. Orders have been introduced from time to time, many at the request of growers themselves, to help restrict the spread of disease from infected areas to those reported to be non-infected. Some of these take the form of prohibition of sale of plants unless they conform to certain conditions of certification or are free from pests and diseases mentioned in a Schedule to the Order.

Officers engaged on work under these Orders are provided with warrants giving them the necessary authority under the D.I.P. Acts, and are expected to produce them on request. They are required to visit auction sale rooms and premises on which plants are exposed for sale, for the purpose of making a check inspection. In the event of any plants being substantially affected by a pest or disease mentioned in the Schedule to the Sale of Diseased Plants Orders of 1927 to 1943, the sale may be stopped. Similar action may be taken in respect of potatoes and narcissus bulbs visibly rendered unfit for planting following attack by pest or disease. Much is left to the inspecting officer's discretion as to when action under the Order is taken, and a reasonably safe guide would appear to be that he should ask himself whether he would plant the affected material. The notice issued by the officer usually prescribes appropriate measures to deal satisfactorily with the case.

Strawberry plants and black-currant bushes may not be sold unless they are the subject of a certificate issued by the Ministry of Agriculture under its relevant certification scheme. The number of the certificate must be given before or at time of sale in writing to the

purchaser. Officers may, where these plants and bushes are exposed for sale, require the certificate number relating to those stocks.

Authority is also given to officers to deal with dead wood in fruit planatations in order to control the Silver Leaf fungus. The Silver Leaf Order further requires the removal of all dead wood from orchards by 15 July of each year, but destruction by fire of all dead wood bearing fructifications of this fungus can be required under the Order at any time.

Action under several other Orders may be exercised; but I have mentioned sufficient to illustrate those efforts to check the spread of serious pests and diseases.

A very important aspect of this work is to be found in the various crop certification schemes. Whilst they do not apply to a large number of crops at present, they have a marked effect on raising the standard of production of those crops and plants to which they are applicable. It would take far too much space to attempt to outline these schemes individually, particularly when explanatory memoranda are available to all interested persons upon application to the Ministry of Agriculture; suffice it to say they apply to black currants, strawberries, raspberries, fruit tree root-stocks, potatoes and hop gardens. Of these, only two are compulsory, viz. black-currant bushes and strawberry plants, made so by the Sale of Strawberry Plants and Black-currant Bushes Order, 1946.

Inspecting officers engaged on these duties are trained to be able to identify a large number of potato varieties: similarly, many commercial varieties of strawberries and black-currants must be identified. This skill has been achieved with the help of the research stations which have prepared 'keys' based on foliage characteristics, wood or stem coloration and general habit. However helpful such keys may be, the officer in the field must be able to recognize variety at walking pace—he cannot afford to stand, key in hand, over a bush or plant—he just knows (or fails to know) and goes on taking count of rogues and making notes on the presence of pest or disease. There are tolerances to which inspecting officers must work in making their recommendations for a 'pass' or 'fail', and these must be applied rigidly to form a uniform standard which is set high with the object of making available propagating material on which a grower can place reasonable reliance, and in all our schemes we rely, as we must do, on the integrity of the raisers of certified stocks.

It is hoped that sufficient has been said to impress upon you that our inspecting officers have to be exceedingly well trained, experienced in the identifications of pest or disease, tactful, and always ready to make a decision to which they are prepared to adhere firmly.

However irksome growers, farmers and importers of plants have found the operation of our Orders and Regulations in the past, I am sure that a very large proportion are sufficiently enlightened to-day to realize that such regulations have been instrumental in greatly reducing the risk of introducing destructive pests and diseases. Furthermore, they have considerably slowed down the rate at which many a serious pest or disease could have spread within our own country.

THE SUGAR BEET EELWORM ORDER 1943

By F. G. W. Jones, *School of Agriculture, University of Cambridge*

The Sugar Beet Eelworm Order 1943 was intended to enforce crop rotation in those fields and localities menaced by the beet eelworm (*Heterodera schachtii* Schm.). The information which led to the issue of the Order was based on work carried out by Mr F. R. Petherbridge, Mr J. H. Stapley, Dr D. Price Jones, Mr K. P. Humphries, Mr B. D. W. Morley and Mr F. G. W. Jones. The work was under the direction of Mr F. R. Petherbridge who was also responsible for most of the negotiations. More recently, Dr H. C. Gough and Mr J. J. W. Williams of the N.A.A.S. have been concerned in the operation of the Order.

Beet eelworm was first found attacking sugar beet in Great Britain in the winter of 1934–5. It is now clear that it had been present for many years previously on mangolds and, in fact,

the first records of its occurrence were on mangolds near Bristol (1928) and near Cardiff (1932). Following upon the appearance of beet eelworm on sugar beet, a clause was introduced into the British Sugar Corporation's contract forms in 1936 forbidding the cultivation of beet after beet or mangolds. Although it did not appear so at the time, this was the first step towards legislation and represented a reversal of the previous policy of beet at any price, a policy necessarily followed to achieve the establishment of the home beet-sugar industry.

In 1937, a field to field survey was commenced in localities where sugar beet had been cultivated intensively. To begin with, farmers with infected fields were given advice on an individual basis but, as the number of infected fields increased between 1937 and 1939, a definite policy was worked out and was accepted by the British Sugar Corporation. Infected fields were delineated on 6 in. Ordnance Survey maps and areas were defined around groups of infected fields and allotted to the nearest beet-sugar factory for administration. Although part of the British Sugar Corporation, the individual factories still operated in the field on a competitive basis, and contracts refused by one factory on grounds of eelworm infection or close cropping, might be accepted by another. Fields within the areas defined (later known as 'special scheduled areas') whether infected or 'clean', were treated as units. In practice, where a field is jointly occupied by several tenants, and cropped in a multiplicity of strips, control of rotation is rendered exceedingly difficult. Furthermore, the first limits to the spread of an infection are the more or less permanent boundaries by which the field is enclosed. The term 'clean' used above requires some qualification. Neither by field inspection nor by soil sampling is it possible to declare a field absolutely free from eelworm. The term 'clean', therefore can be taken to mean 'substantially free' or 'not known to be infected'. Within the 'special scheduled areas' sugar beet was to be grown on a three-course rotation on 'clean' fields and on a four-course rotation on slightly infected fields. Heavily infected or 'sick' fields were first to be given a rest of 5 years from susceptible crops, after which, a soil sample was to be taken to ascertain that the infection had fallen to a safe level and, this being so, the field was then classed as slightly infected and a four-course rotation followed thereafter.

The possibility of obtaining an Order was first considered in 1939. By this time the weakness of the factories' position was becoming clear. Factory contracts afforded no real control over mangolds and the various Brassicae, also susceptible to attack. A contract might prevent the cultivation of beet after mangolds but could not prevent the cultivation of mangolds after beet. Moreover, the factories had no control over the sale of seed potatoes or transplants from infected land and could not take any reasonable steps to limit extension of beet eelworm spread.

In the first proposals for the Order a bold step was taken. A large slice of Black Fen soil was declared to be menaced by beet eelworm and boundaries to this area were drawn enclosing portions of Huntingdonshire, the Isle of Ely and west Norfolk, together with corners of Cambridgeshire and west Suffolk. This area, as well as a number of infected sewage farms, the most important being a large sewage farm in the heart of Norfolk, were to be scheduled and within these scheduled areas the policy already outlined was to be enforced. A period of argument and negotiation followed culminating in the issue of the Order in 1943. The negotiations were conducted through the Committee for Sugar Beet Research and Education which had financed the survey work. The Committee included representatives of the National Farmers' Union, the British Sugar Corporation Ltd., the Agricultural Research Council, the Ministries of Agriculture and of Food as well as specialist advisers and other persons. The Committee were convinced of the desirability of legislation and were able to speak with one voice to the appropriate branch of the Ministry of Agriculture.

Meanwhile surveys were being continued and new infections were being recorded at the rate of 100 or more per year. In 1938, 96 infected fields were known, by 1943 the figure had risen to 556 fields. In 1944, the scheduled area was extended by additions to the main area and the delineation of a new area lying north of Peterborough and extending into the western corner of the Holland Division of Lincolnshire. At present (1950), the number of infected fields cannot be far short of 1000 and new centres have appeared in a number of localities.

With the issue of the Order, the policy already outlined was implemented in a rather ingenious manner, through a system of licences to be issued by War Agricultural Executive Committees. The policy itself was not declared, so that changes might be made as occasion demanded. In addition, infected land came under the Order wherever it might be found and there were provisions for dealing with the sale of transplants or seed potatoes from infected land. One stumbling block remained. The Ministry of Agriculture would not accept the field as a unit and infection referred only to the actual land on which it was found. Only recently has this point been conceded and the person determining the infection may now define the limits within which it is deemed to occur.

A meeting of representatives from all War Agricultural Committees and beet-sugar factories concerned was convened and the policy outlined was accepted as a basis for the issue of licences. The field was accepted as a unit regardless of the terms of the Order, but only in those cases where an actual infection had been found. During the period that followed the issue of the Order, difficulties arose from variations in the vigour with which the Order was enforced and in the interpretation of its terms. These variations were apparent to farmers with land lying near or across county boundaries. Only in Norfolk where an 'eelworm officer' was appointed was the Order properly enforced. The administrative difficulties of the Ely Beet-Sugar Factory with an area extending into five counties can well be imagined. Apart from the counties, the factories themselves were not all equally meticulous in operating the Order and difficulties were further increased by the dissolution of the old Advisory Service and the institution of the National Agricultural Advisory Service, which resulted in wholesale changes of personnel. Within the last year (1949–50) determined efforts have been made to retrieve the position and, as the outcome of negotiations at various levels, the other counties concerned are to follow the example of Norfolk and appoint 'eelworm officers'.

The main technical problems in the operation of the Order spring from strip cultivation and from the large range of crop plants susceptible to beet eelworm. These technical difficulties, great as they are, hold second place to the human factors. It is not possible by legislation alone to coerce a large section of the community into doing what may be thought best for it. Legislation can only be used in a few cases as a 'big stick' to beat the more awkward individuals into submission. Even so, the fines and the stigma involved are small compared with the profits made. Compliance with legislation is to be obtained through persuasion, education and explanation. In this, talks, lectures and the actual field surveys can play a big part.

On the continent and in the United States of America, it took about 50 years for the beet eelworm to become widespread from the time when infections were first noted. A similar period has elapsed in Great Britain from the finding of the potato root eelworm up to its present widespread dispersal in the main potato growing areas. If the dispersal of beet eelworm here follows a similar course, all the important beet growing areas will be extensively infected by about 1980. Rotation, whether voluntary or enforced, cannot prevent the further spread of beet eelworm but it should reduce the rate of spread somewhat and, more important, prevent serious crop losses. The time thus gained ought to be fully utilized in the prosecution of fundamental and applied research that may lead to the formulation of more direct measures of control.

THE PROGRESSIVE WILT OF HOPS ORDER

By W. G. Keyworth, *East Malling Research Station*

This order, which came into effect on 1 January 1948, is designed to combat the spread of the very severe 'Progressive' type of Verticillium wilt of hops.

This disease is caused by a virulent strain of the fungus *Verticillium albo-atrum* Reinke & Berth. The strain is believed to be a mutant from the normal *V. albo-atrum* and is thought to have arisen on one farm near Paddock Wood, Kent, about 1930. Since that time the disease

has spread to over 100 farms in the important Weald hop area. The fungus grows in the soil and enters the hop plants through their roots, then invading the stems and finally growing into the leaves. The plant is killed and the dead leaves fall off and are carried about by cultivators or blown in the wind, thus infecting the soil in the same field or nearby ones. Infected cuttings and rooted sets also act as agents of spread and constitute a great potential danger to hop areas (e.g. in the west Midlands) which are as yet uninvaded.

The first sign of a progressive outbreak is usually one or two dead or dying plants. From these the disease spreads year by year until after a few years most of the hop field may be useless. The ordinary commercial hops cannot be planted on infected ground and thus, since hop fields give an annual return of some £500 the financial loss is considerable. Replanting with new resistant varieties is proving a successful means of reclaiming wilt areas but, in addition, measures to check the disemmination of inoculum are essential.

With this end in view, the Order requires that the grower shall notify any outbreak of progressive wilt and shall burn all infected stems and leaves. He is also prohibited from selling any planting stock from an affected farm.

In some cases it may be difficult to decide whether a new outbreak is of the progressive type or is caused by the normal strain of *V. albo-atrum* which produces a mild 'fluctuating', outbreak of little economic significance. Attempts are now being made at East Malling to devise a laboratory test to differentiate between the mild and virulent strains of the pathogen. Some success has been achieved with empirical methods using growth characters on modified Czapek-Dox agar, but these tests are not yet reliable enough for accurate diagnosis.

SOME SCIENTIFIC ASPECTS OF THE CERTIFICATION SCHEMES FOR FRUIT PLANTS

By R. V. Harris, *East Malling Research Station*

Positive certification for health

The first certification schemes for fruit, the so-called Ordinary Certificates for black-currants, strawberries and raspberries, primarily cover varietal purity, with the proviso that stocks receiving a certificate *appear* free from diseases at the time of inspection.

The recently introduced Special Stock Certification schemes for strawberries and raspberries and the scheme for hops are primarily concerned with the maintenance of health and productivity. This shift of emphasis is demonstrated by the 'Sales of Strawberry Plants and Black-currant Bushes Order, 1946' which was introduced entirely on health grounds, to counter a repetition of the decline of soft fruit stocks occurring after the 1914–18 war, and which, by forbidding the sale of any but officially approved or certified planting stocks, virtually converted the voluntary certification schemes for these crops into compulsory ones. The continuance of this Order and its possible extension to other crops is the subject of much present controversy. Are the growers who wish to purchase stock entitled to an official assessment of its health? This raises the question of the validity and adequacy of the health criteria upon which such assessment is based.

Here a clear distinction emerges between compulsory approval introduced: (1) as an emergency measure at a time when demand for stock greatly exceeds the reliable sources of supply and designed to protect growers from the more degenerate stocks; and (2) as a permanent system of health certification according to positive graded standards of health and productivity. The trend of the former, as illustrated by the existing Order, is to vary the average standard of approval adopted in order to suit the supply of stock to the demand. A *sine qua non* of the latter system, if it is to capture, by the consistent excellence of its products, the confidence and, ultimately, the voluntary support of the consumer and propagating industries as a whole,

is an adequate scientific basis for the diagnosis and control of the diseases relevant to stock degeneration. In the case of strawberries and raspberries the latter are now known to be complex virus diseases, and recent advances in research on these quite clearly indicate the essential features of such a system.

The scientific basis for a health certification system

This briefly is as follows:

(1) The economic strawberry and raspberry virus diseases are composite in structure and are only graft and insect transmissible. Identification is therefore limited by the sensitivity-range of the host indicators available.

(2) No completely immune varieties have been discovered, and varieties and host species vary greatly in their levels of tolerance to severe virus combinations.

(3) Varieties and species differ widely in their symptom expression of viruses individually and in combination. In the case of some raspberries, degeneration may occur in the absence of diagnostic symptoms.

(4) Expression in certain instances fluctuates with changes in plant environment, seasonal and otherwise.

(5) The viruses vary widely in their relations to insect vectors.

(6) Raspberry varieties differ in their sensitivity to infection by means of insect vectors.

It will be evident from these facts that the information on precise health status to be derived from one or two annual field inspections is very limited in character. The machinery of certification should therefore be correspondingly elaborated as follows.

Basic requirements and structure of a system of health certification

(1) Stock to be certified should be derived from 'nuclear' or foundation plants selected both on direct tests for virus content, and on horticultural merit.

(2) The accumulation of non-expressed but important component viruses must be checked by limiting the eligible stock-harvests from any one issue of tested 'nuclear' stock to one, two or three, etc.

(3) Ample provision should be made for the early selection, preservation from infection and later incorporation into the scheme, of new varieties of merit.

(4) Propagation of planting stock must be effected, not as a by-product of cropping plantations, but in special nursery plantations designed to facilitate efficient inspection and disease elimination.

A system embodying these points comprises the three following stages (Harris & Cadman, 1949):

Stage I. (*a*) Trial and selection of varieties to be propagated under the Scheme; (*b*) early calibration of short-listed varieties for virus reaction; and (*c*) selection and preservation under vector proof conditions of a 'nucleus' with minimum virus content.

Stage II. (*a*) The annual production and issue of a nucleus of the order of about 100 plants of each certifiable variety, from the basic stocks of stage I(*c*); and (*b*) the multiplication of this annual issue to a level sufficient to supply commercial propagators of certified stock, under specified conditions of isolation, etc.

Stage III. Production of a limited number of certifiable harvests from each issue of nuclear stock (stage II) under cultural conditions laid down by the certifying authority.

Stage III is clearly the field of the nurseryman and grower-propagator entirely. Stages I and II provide him with the foundation material of known health status which he is to convert to bulk supplies of certified stock adequate to the needs of the fruit growing industry. The present close inter-relation of the operations of stage I and the work of research centres is sufficiently evident.

In conclusion, it is suggested that the obviously non-profit making functions comprised by stages I and II are as basic and integral to this system of health certification as are the field inspection and other services included in stage III. It follows that the actual production of nuclear or foundation stocks should be regarded as a function of the certifying authority, and as such entitled to support from public funds.

REFERENCE

HARRIS, R. V. & CADMAN, C. H. (1949). Can the health of raspberry stocks be improved? *Scot. Agric.* **28**, 194.

MARKING AND BREEDING *TESTACELLA* SLUGS

BY H. F. BARNES AND BARBARA M. STOKES, *Rothamsted Experimental Station*

(With 4 Text-figures)

I. GARDEN STUDIES (H.F.B.)

Introductory

Testacella is a most interesting genus of slugs. The species are predacious, living on earthworms, whereas other slugs are scavengers and plant feeders; their alimentary system is straight, not twisted on itself as in other slugs; and they possess a small external shell. Two species, namely *T. scutulum* Sowerby and *T. haliotidea* Draparnaud, out of the three British species occur in Bedford gardens.

Activity and weights

These species occur in quite small numbers, the largest number so far seen in an half-hour period has been only twenty-nine. But in spite of this an attempt has been made since September 1946 to study their activities by means of the half-hour sampling after-dark method previously found to be satisfactory for other slugs (Barnes, 1944; Barnes & Weil, 1944, 1945). The numbers of *Testacella* seen active on the surface of the soil in one garden (27 Rothsay Road) have been observed throughout a number of half-hour periods (totalling 137 hr.) and the individuals have been recorded as 'just hatched', 'very young', 'small', 'medium' and 'big' as a rough indication of their size. Parallel half-hour samples have been taken in the adjoining garden (no. 25), but in these cases the slugs (294 in all) have been removed, weighed individually to the nearest 0·01 g. and not returned to the garden. Using these weights it appears that 'just hatched' weigh 0·01–0·02 g., the 'very young' about 0·03–0·06 g., while the 'big' weigh about 0·8–2·94 g. or more. Because on many occasions no *Testacella* have been seen or found, it has not been possible to construct activity curves for the years separately, but the numbers seen in one garden in each month of the 4 years have been treated together. Similarly, the weights of the slugs removed per month from the next garden over the 4-year period have been dealt with together. It is not possible to separate for certain the smaller specimens of the two species, neither is it always possible to distinguish the larger specimens when using a hand-torch because of the yellowness of the light, although by daylight this is usually possible and colour photographs reveal this slight colour difference. For these reasons the figures relating to the two species have been considered together.

Fig. 1 shows the average numbers seen on the surface per half-hour observation each month of the year in the garden of 27 Rothsay Road. This indicates that, in spite of the fact that the number of observations were not the same for each month of the year nor for one year compared with the next, it is comparatively unusual to see a single *Testacella* in a single half-hour's observation during January, February, March, July and August. It will be seen that this statement is based on a considerable number of observations in the first 3 months of the year

but only on a small number in July and August. Actually no *Testacella* have been seen in this particular garden during August, but in other gardens *Testacella* have occasionally been seen in this month. A small peak in numbers is indicated in May but the largest numbers clearly occur in November. This figure also shows the average weight of *Testacella* collected per month in the next-door garden. The average weights for February, July and August do not fit the curve, but so few individuals were collected in these months that the average figure is unreliable. Although the average weights in April and May were only just over 0·9 g., the heaviest individuals were actually 2·94 and 2·82 g. respectively. Conversely, the average weight for November was 0·13 g., but out of the fifty-three individuals weighed in November twenty-six weighed only 0·01 g. The data show, with the exception again of those for February, July and August, that newly hatched and large specimens may be seen in each month of the year, but that there is an annual rhythm in the relative numbers. The peak of newly hatched slugs occurs in November, while that of the large ones is in April and May. The only clue to mating has been the observation of 24 May 1948 when two large individuals were seen apparently following each other on a moss-covered lowest brick of a wall. Clusters of eggs of *T. scutulum* have been found by digging during May. They were found at varying depths down to 14 in. Eggs were discovered in each of the six holes dug and a total of sixty-nine were recovered.

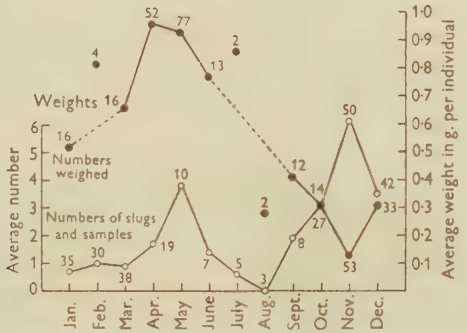


Fig. 1. Average monthly numbers and weight of *Testacella* spp. at 25 (weight •—•—•) and 27 (numbers o—o—o) Rothsay Road, Bedford, September 1946–September 1950. The numbers of samples on which the average numbers are based and the numbers of individual weighings on which the average weights are based are shown.

Marking

These garden observations again emphasized the desirability and necessity, if one is to assess the total population in an area and not merely those active, of studying the behaviour of the individual rather than the mythical average specimen. Since there is a small external shell on these slugs it was decided to attempt marking with cellulose acetate paint. Accordingly, the shells of eighteen individuals were painted red and the slugs were liberated on 15, 16 and 17 October 1948 while a further eleven were similarly painted white and liberated on 25 October the same year. A few of the marked individuals were seen that October and November and one in December; one red-marked slug was seen the following February, April and October, while white marked individuals were seen on four occasions in February, March and May that year. In 1950 the red-marked *T. haliotidea*, that had been seen during October 1949, was seen again on 21 March and for the last time again on 10 May, 570 days after marking it. A red-marked *T. scutulum* was seen on 21 May, 581 days after marking and it then weighed 1·31 g. In another experiment three slugs were painted green, five yellow and three blue, and all these were liberated on 22 May 1950. A few of these have been seen

again. This method of marking has thus been partially successful, but it is not possible to determine on how many individuals the mark remains and in any case it is really mass and not individual marking.

Time of activity and extent of movement

An experiment was designed to find out the time of nightly activity, the duration on the surface, the distance travelled, etc. Part of a rose bed was divided into 6 in. squares by strings passing slightly above the soil (Fig. 2). In this way it was possible to plot the position of the slugs as they appeared on the surface. Observations starting 1 hr. after sunset were made at half-hourly intervals throughout the night 27–28 May 1950 when the sun set at 9.1 p.m. and rose at 4.52 a.m. All times are given in B.S.T. The positions of the slugs were charted. The

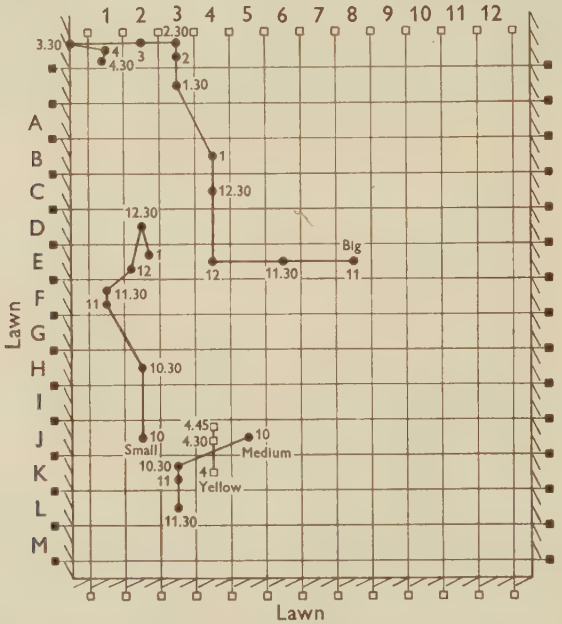


Fig. 2. Movement of *Testacella*, 27–28 May 1950, sunset 9.1 p.m.; sunrise 4.52 a.m. B.S.T. The position of four individuals at different times of the night are given. The squares are 6 in. each side.

number of *Testacella* seen at each observation varied from three to eleven; four were on the surface at 10 o'clock, eleven at midnight, while the three seen at 4.45 a.m. when it was again quite light were in various stages of re-entering the soil. The meanderings of a 'small', a 'medium', a 'big' and a yellow-marked individual are shown in Fig. 2. From this it can be seen that it took between 30 and 60 min. for an individual to move 1 ft., one slug in fact traversed about 7 ft. in the 5 hr. it was on the surface. In many cases the slugs were only seen on the soil on one occasion and apparently stayed on top of the soil only a very short period. There was no evidence on this particular night, when the air temperature nearby fell from 50·5° F. at 10 o'clock to 47° F. at 4.30 a.m., that there was most activity either at sunset or at the sunrise twilight. There was a hint that most *Testacella* were on the surface $1\frac{1}{2}$–$2\frac{1}{2}$ hr. after sunset. All sizes appeared, but only one marked slug was seen although twelve such

individuals (eleven of which had been liberated only 5 nights previously) were thought to be in the area studied. In no instance during this night was a *Testacella* seen to be eating an earthworm, although the feeding process has been seen several times during the 4 years' observations. This has been described elsewhere (Barnes, 1950).

II. Laboratory studies (B.M.S.)

Method

It is not easy to keep a constant watch on the activities of *Testacella* slugs when underground. The following method used for breeding was evolved in order to overcome this difficulty, keeping as near to natural conditions as possible.

In November 1949 eight specimens of *T. haliotidea* and *T. scutulum* were obtained for individual study. Each after weighing was kept in a corked glass tube 4 in. × 1½ in., filled up to two-thirds of its height with ordinary damp garden soil. A living earthworm about 2 in. long was added to each tube. The tubes were kept in a dark, underground cellar.

Daily inspections, when the cork was removed for airing the tube, showed if the slug had eaten the worm. If so, one more worm was given. Uneaten worms were removed only if they appeared unhealthy, when a new one was substituted. The tubes were washed out once a week and the old soil replaced by fresh. On the same day each week a routine weighing of each slug was made to record growth rates.

Besides these individual slugs, some were kept together in soil containing several worms in large glass vessels in order to provide specimens whenever required. These jars were also inspected daily.

Growth rate and feeding

In *T. scutulum* an increase in weight occurred during the period November to April (Fig. 3). This corresponds with the garden studies when most small ones were found in November and most large ones in April and May (Fig. 1). Mating occurred in four individuals when they weighed 1·35, 1·37, 1·38 and 1·9 g. respectively. At the commencement of egg laying these weighed 1·58, 2·17 and 2·08 g. respectively, the fourth slug dying on 13 March without laying eggs. Another individual began laying when it weighed 1·26 g. There appears to be a tendency to lose weight and appetite in the autumn, but whether this is a prelude to death is not yet known. Taylor, in his monograph (1907), mentions that these slugs may attain 5 or 6 years of age.

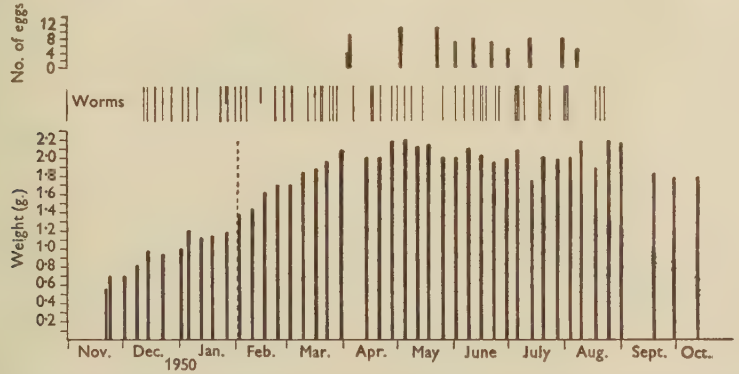


Fig. 3. Growth, feeding and egg laying of *T. scutulum*. The dotted line indicates the date of mating. One line represents a worm, half a line represents half a worm.

Feeding is a leisurely process performed with tentacles withdrawn. Whole or half worms are eaten on and below the soil surface. The worm is gradually sucked in, in large gulps with intervening pauses. Rapid regurgitation is not uncommon either as a result of overeating or being handled too soon after feeding. After a meal, the slug assumes a quiet characteristically humped position.

Under the conditions described, feeding is spasmodic, and after a meal several days usually elapse before another worm is eaten (Fig. 3). One *T. scutulum* averaged a worm a week over 11 months. Species of earthworm known to be eaten include *Allolobophora caliginosa*, *A. chlorotica*, *Octolasium cyaneum*, *O. lacteum* and *Dendrobaena subrubicunda*.

Eggs and hatching

The first mating pair of *T. scutulum* was seen on 21 January 1950 in one of the jars containing several slugs. They were under the soil surface near the bottom of the jar. The genital aperture of *Testacella* is at the right of the head and the two slugs were seen touching, motionless, head to head and joined by mucus. After 20 min. they separated, but the full duration of the mating process has not yet been discovered. The slugs were then weighed and each placed in a separate tube of soil with a worm. Two more slugs were seen to mate on 2 February and were dealt with in a similar manner. The three which lived began to lay eggs 54, 61 and 95 days after mating. Eggs were also obtained from two other slugs which were given the opportunity of mating, though the process was not observed.

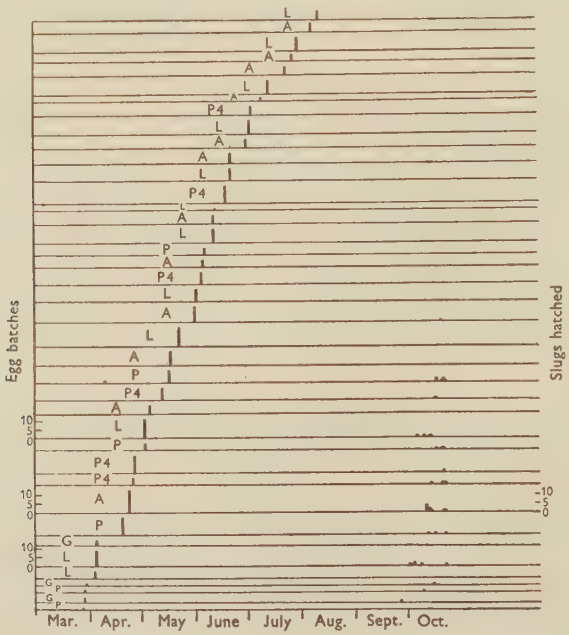


Fig. 4. Egg batches and hatching of *T. scutulum*. Batches laid by slugs G, A, L, P and P4 are indicated.

Normally the eggs are laid in batches, from two to thirteen at a time but occasionally a single egg is laid (Fig. 4). Unlike the eggs of other slugs such as *Arion* and *Limax* spp. those

of *Testacella* spp. are not gelatinous and translucent but possess a brittle calcareous shell. When new-laid they are white but turn light brown after a few hours. Oval in shape, those of *T. scutulum* measure 4 × 3 mm. and weigh between 0·01 and 0·02 g. while those of *T. haliotidea* are 7 × 4 mm. and weigh 0·05 to 0·06 g. each. In both species the eggs fall in a cluster as they are laid and only when the batch is complete does the slug move away. The process may occupy several hours depending on the number of eggs in the batch.

Taylor (1907, p. 4) mentions that the eggs explode with a perceptible crack when placed on the hand or in a warm place. This is true. New-laid eggs of *T. scutulum* were tested in the hand, some shattered while another exploded with force, the pieces landing 9 in. away. The noise is sharp and quite audible.

After the first egg batches obtained in March and April, each slug laid further batches without mating again, at intervals varying from 3 to 27 days. The last batch was laid on 12 August (Fig. 4). Thus eggs were produced in the 6 months, March to August. The highest total number of eggs laid by one slug was 84; the total for five slugs being 208. Two slugs which laid all through the summer produced ten and eleven batches respectively.

The eggs were kept in the dark cellar, some on damp sand, others in or on damp soil while a few were placed in tubes of soil buried in a box of peat outside. Since none had hatched by the end of August some eggs were opened and living embryo slugs were found with a small shell on the posterior end.

The first *T. scutulum* hatched on 27 September and others followed. The length of time between laying and hatching varied between 144 and 201 days, as eggs laid in March–June all hatched in October (Fig. 4). This is much longer than the 20 to 36 days mentioned by Taylor (1907, p. 16).

In hatching, a small hole is made at one end of the egg revealing the tail end and shell of the slug. Sometimes as much as 2 days elapse before the slug moves out. The actual exit has not yet been seen. Egg shells are left intact except for the one hole. The young slug is white with an almost transparent shell and varies in length from 4 mm. when contracted to at least 7 mm. when extended. It is active and eats small earthworms in the same manner as the larger specimens.

REFERENCES

Barnes, H. F. (1944). Seasonal activity of slugs. *Ann. appl. Biol.* **31**, 160–3.

Barnes, H. F. (1950). Worm-eating slugs. *Bedfordshire Naturalist*, **4**, 24–6.

Barnes, H. F. & Weil, J. W. (1944). Slugs in gardens: their numbers, activities and distribution. Part 1. *J. Anim. Ecol.* **13**, 140–75.

Barnes, H. F. & Weil, J. W. (1945). Slugs in gardens: their numbers, activities and distribution. Part 2. *J. Anim. Ecol.* **14**, 71–105.

Taylor, J. W. (1907). *Monograph of the land and freshwater Mollusca of the British Isles.* Vol. 2: Testacellidae, Limacidae, Arionidae. Leeds.

TECHNICAL AND BIOLOGICAL ASPECTS OF INSECT DRIFT

By C. G. Johnson, *Rothamsted Experimental Station, Harpenden, Herts*

The paper described old and new methods of high altitude trapping and the ecological approach to the study of drift. Work at Rothamsted and Cardington was in a transitional stage where many data, accumulated with a now obsolete method, had given suggestive results which now required verification and extension by the use of the more accurate suction trap. Emphasis was laid on the importance of regarding drift in its context with terrestrial ecology, particularly with the flight behaviour of insects at crop level, on which the initial source of supply to the upper air depends. The integration of density changes in and over the crop with conditions of turbulence and convection was seen as a central feature in the study of vertical distribution and of horizontal drift.

THE PREFERENCES SHOWN BY HONEY-BEES FOR CERTAIN NECTARS

By Gwenyth R. Wykes, *Rothamsted Experimental Station, Harpenden, Herts*

When gathering nectar, honey-bees appear to exhibit marked preferences for the flowers of certain species, whereas they do not visit other plants which are flowering at the same period. Various investigations have been carried out to determine the factors which may possibly affect this selective nectar-gathering behaviour. Several workers have established that both sugar concentration and volume of nectar influence the relative attractiveness of different nectars to bees. To determine whether variations which were found to occur in the constituent sugars of nectar may be a contributory factor influencing the selection of certain nectars by honey-bees, a series of field and laboratory experiments was carried out during 1949–50. In both types of experimental series, an equal volume of solutions of the same total sugar concentration for any one experiment, but of different sugar composition, was made available to a large number of bees, and their average relative preferences for solutions of different single sugars, or mixtures of sugars, determined. In the field experiments, counts of the number of bee visits to dishes containing different solutions were made over an extended period, and in the laboratory experiments, measurements of the uptake of different solutions by bees were made. It was found that the sugars which occur in nectar are not equally attractive to bees, for consistent preferences were shown for certain solutions. Certain apparent anomalies occurred in the relative preferences shown for solutions of mixed sugars compared with the preferences for their constituent sugars in single solution; and the relative preference appeared to change with changing concentration. The evidence from these exploratory experiments suggests that sugar composition, in addition to sugar concentration and volume of nectar, may partially influence the selection of certain nectars by honey-bees.

THE EXPRESSION OF SYMPTOMS OF LEAF-ROLL VIRUS IN POTATOES

By J. H. Wilson, *Department of Agriculture, Tasmania**

In the course of growth analysis studies of healthy and leaf-roll infected potato plants the effects of nitrogen, phosphorus, potassium and shading on the expression of leaf-roll symptoms were observed. It was found that high nitrogen, in the presence of phosphorus, particularly during early growth, gave the conditions most favourable for masking of symptoms. Reduction of light intensity by shading had only a slight masking effect, but it seemed likely that with more intense shading, the relative importance of this factor would have been increased. Symptoms were slightly intensified by the addition of potassium.

In a later experiment the interaction of variety with nutrition in relation to leaf-roll symptom masking was studied. A tolerant variety, Up-to-Date, was included in addition to the intolerant variety, Craig's Defiance, previously used. A basal dressing of phosphorus and potassium was given to all plants and the level of nitrogen was varied. In Craig's Defiance the addition of nitrogen significantly reduced the severity of symptoms as in the previous year's experiments, but masking was by no means complete. In Up-to-Date the effect was much more striking. Symptoms were completely suppressed until nearly flowering time, and were then of only mild intensity and confined to the basal leaves. Under conditions of low nitrogen, symptoms were very severe in both varieties.

There is a good reason to suppose that these findings apply equally well to plants grown in the field where, in fact, conditions may be even more favourable for symptom masking. High

* At present working at Rothamsted Experimental Station, Harpenden.

levels of nitrogen and phosphorus, while suppressing early symptom development, would tend to produce vigorous growth. This would mean that the basal leaves, in which symptoms might eventually be expected to develop, would be in dense shade and hence subjected to the masking effect of this additional factor.

It seems, therefore, that under favourable field conditions, and given a tolerant variety, symptom development may be very much delayed or even completely suppressed throughout the season. This is of considerable importance in relation to seed certification in which success depends on the recognition of infected plants in the growing crops.

THE EFFECT OF MICROCLIMATE ON THE ESTABLISHMENT OF TIMOTHY (*PHLEUM PRATENSE*)

By Stella S. Williams,* *Grassland Research Station, Stratford-on-Avon*

The campaign instigated by Sir George Stapledon and the wartime plough-up policy have resulted in a considerable acreage of our hitherto old pasture being given over to leys or temporary grass. The success and rapidity with which new swards can be established is therefore of considerable economic importance. Many of these leys are sown with simple mixtures, frequently consisting of one grass and white clover (*Trifolium repens*). Of the mixtures in common use, ryegrass (*Lolium perenne*) and white clover is the easiest to establish and usually gives a good take independent of meteorological conditions, while timothy (*Phleum pratense*) and white clover comes at the other extreme, timothy being sensitive to weather conditions and difficult to establish.

I have been studying the establishment, from spring sowing, of these two mixtures over four seasons, in particular trying to find the reasons underlying frequent poor takes of timothy. The results indicate that the most serious causes of seedling casualties in this species are drought and heavy early grazing.

It was noticed that seedling mortality during drought periods was lower where the seedlings were growing in proximity to larger oat plants; the latter apparently provided shelter. Similar protection was given by white clover and even by timothy seedlings themselves when growing close together. Some of the apparent value of such shelter is later lost, as the greater the initial density of seedlings the more they will suffer from competition later. This does not preclude the importance of protection at the early critical stage, which may prevent a failure. It does not matter if the seedling population exceeds any possible adult population: oats will quickly die if well grazed and timothy and white clover will adjust themselves through competition to a population the area can maintain. A wrong balance between these species can be largely rectified later by management and manuring. In 1949 this *protection/competition* relationship for timothy, sown in pure sward with oats cover, worked out so that the highest percentage survival occurred in areas that had an initial seedling density between 120 and 160 seedlings per square foot; lower densities suffered from exposure and higher densities from competition. The initial density corresponding to the highest survival will vary with the type of soil, the time of sowing and meteorological conditions.

Tillering seedlings do not normally die from drought, although their growth rate may be reduced. Younger seedlings are more susceptible but in a moderate drought they can remain alive in a state of arrested growth for some time (Chippindale, 1948); if conditions improve growth will restart, but the critical phase will have been prolonged and the competitive power of the seedling against other less susceptible species reduced. Heatwaves of up to a week or 10 days' duration, therefore, do not cause many fatal casualties, longer ones do.

Why are there fewer casualties among seedling timothy plants growing fairly close together or in proximity to either oats or white clover than among sparsely spaced seedlings surrounded by bare ground? Thinking in terms of water supply the greater the plant density,

* Née Champness.

the greater the quantity of water removed to supply transpiration needs. The roots of the dying timothy seedlings, however, have usually penetrated less than an inch into the soil and so lie in the area that is yielding moisture through bare ground evaporation, while the roots of the oats and clover grow more quickly and tap a deeper layer for transpiration. It is therefore possible for these plants to shelter the top layer of soil from the drying effects of wind and insolation, without competing for the limited moisture present in this layer. It is more difficult to see how a high density of timothy seedlings themselves can help to conserve moisture in the top soil around their own roots; sometimes it may be explained by one or more plants gaining ascendency and so protecting their neighbours.

Soil-moisture determinations made during the 1949 drought have shown that the seedling cover did effectively reduce evaporation from the soil surface and aided the conservation of moisture in the upper layers (Champness, 1950).

Day temperature records were made in swards of varying states of development. Bare ground typical temperature gradients, characterized by an increase in temperature near the soil surface, were obtained. Very young seedlings modified this gradient by reducing the intensity of heating-up near the soil surface. Older tillering seedlings have their own 'active surface' or region of heating-up where insolation strikes the leaf surfaces, but for some time sufficient rays still penetrate this barrier to produce a second, but reduced region of heating-up near the soil surface. At about the time the sward attains 50 % coverage it changes from being predominately bare ground and becomes a mosaic of sward containing pockets of bare ground. This change appears to coincide with the end of heating-up at the soil surface over a large proportion of the sward mosaic. The temperature gradients from then on show an active surface only in the region of the outer leaf surfaces, except in poor patches. This change over seems to end the critical phase of establishment, from then on it is secure under normal circumstances; this stage is similar to the 'lateral stoppage' described by Ramdas (1946) and appears to be accompanied by an increase in growth momentum.

I have been unable to do any experiments to determine whether moisture or temperature acts as the limiting factor in seedling mortality. The moisture figures quoted are low enough to cause death, while it is doubtful whether the high temperatures recorded (90–95° F.) would cause more than a temporary disorganization of the metabolic processes (Brenchley & Singh, 1922; Brown, 1939), but to seedlings already in a precarious condition these temperatures might be fatal.

Environmental factors are closely interrelated, thus temperature differences within a sward may be taken to indicate a different micro-environment (Geiger, 1927; Champness, 1950). The temperature gradients described indicate, therefore, a gradual modification of the environment as the sward develops. The earlier a good cover can be obtained the less likely are the effects of drought to be serious. This is a case for both early sowing and adequate seed rates. The results suggest 20–25 lb. of timothy seed per acre when sown in pure sward under a cover of oats and less when sown with white clover.

These experiments have been made on the heavy lias clay at the Grassland Research Station: this soil is liable to crack in hot dry weather. When large cracks occur the soil in the vicinity dries out rapidly, often to a depth of several inches and nearby plants may be quickly desiccated leaving a network devoid of vegetation. The cracks tend to follow the lines of least resistance and to develop where the soil is dryest; hence they tend to occur where there are already few plants. In this way initially poor patches become poorer. If it is possible to obtain a good cover before severe heat-waves occur, soil cracking and thus secondary casualties occurring in the wake of the cracks will be reduced.

REFERENCES

Brenchley, W. E. & Singh, K. (1922). Effect of high root temperatures and excessive insolation upon growth. *Ann. appl. Biol.* **9**, 197.

Brown, E. M. (1939). Some effects of temperature on the growth and chemical composition of certain pasture grasses. *Mo. Univ. agric. Exp. Sta. Res. Bulletin* no. 299.

Champness, S. S. (1950). Effect of microclimate on the establishment of timothy grass. *Nature, Lond.*, **165**, 325.

Chippindale, H. G. (1948). Resistance to inanition in grass seedlings. *Nature, Lond.*, **161**, 65.

Geiger, R. (1927). The climate of the layer of air near the ground. Translation from *Das Klima der bodennahen Luftschicht*. Braunschweig.

Ramdas, L. A. (1946). The microclimates of plant communities. *Indian Ecol.* **1**, 1.

REVIEWS

Plant Viruses and Virus Diseases. By F. C. Bawden. Pp. 335, with 60 illustrations. Waltham, Mass.: Chronica Botanica Co.; London: Wm. Dawson and Sons Ltd. 3rd ed., revised 1950. Price 45*s.* or $6.

The third edition of Mr Bawden's book claims to be 'largely a new book' rather than another edition. Comparison with the 1943 edition not only confirms this but impresses the reader with the extent to which virus research has developed during the last decade. Some views which were controversial in 1942 are now either finally rejected or widely accepted and newer controversial theories have been built on them. New virus diseases have been studied and have either found their place in the accepted scheme or revealed the limitations of our concepts.

Although the author states that his is not a text-book, this is true only in the sense that he does not attempt to list and describe virus diseases. It is, in fact, a text-book of virus research, a subject which has expanded so rapidly that it seems improbable that a single volume could cover adequately the range of sciences involved. Yet Mr Bawden has achieved this in a very lucid form. This may well be the last comprehensive book on plant viruses, though not, we hope, the last edition.

The virus specialist and the worker in other realms of biology will react differently to this book. The entomologist or the physiologist, for instance, may be impressed by the volume of factual knowledge relating to plant virus diseases, since viruses are so often regarded as vague nonentities. The virologist, on the other hand, will realize that the bulk of the positive information relates to a comparatively small number of viruses which infect either beet, cucumber, potato, tobacco or tomato and their close relatives. Moreover, the author shows frequently where important gaps occur in our knowledge and stresses the gulf between fact and supposition.

All aspects of virus research are reviewed—symptoms, transmission, vectors, strains, serology, methods of assaying and of purification, chemical and physical properties of virus preparations, crystallinity, particle sizes, inactivation, taxonomy, host-plant physiology and control measures. The book concludes with a provocative chapter on 'Speculations on the Origins of Viruses'. The type and illustrations are clearer than those of the second edition and the indices have been well revised, though the author's own name is curiously absent from the author index.

To the reviewer one of the most interesting changes from the previous edition is the *Addendum* to the chapter on viruses and their vectors. Mr Bawden now accepts recent evidence that at least one virus multiplies in its insect vector, although he has previously argued that the evidence then available was inconclusive. Once the concept of virus multiplication in the vector is accepted, not only does the interpretation of latent periods and long persistence in the vector become more plausible, but also a new field of research is opened. If a plant virus can be cultured in an animal tissue, the possibility of studying an animal virus in a plant host becomes less remote. Virus multiplication in the blood of the insect vector would be more easily understood by the reader, however, if the author had mentioned that insects apparently do not produce antibodies.

The chapter on 'Taxonomy of Viruses' deals with a subject about which there is little agreement among virus workers except that most would concur in the need for some system of classification if confusion is not to be confounded by the increasing number of viruses being studied. There is much in favour of the author's proposal that a start should be made with the large number of viruses and strains which have been studied by serological and immunological methods. The important point is that any system adopted should be based

on the properties of the viruses and not on those of their hosts, even though many viruses must be excluded until more is known about them.

When plants are infected by one of the viruses which are not transmissible by inoculation with expressed sap, there is at present only one method of comparing their virus concentrations. The proportion of transmissions obtained with vectors under comparable conditions, though admittedly crude and giving a measure of 'available' virus only, is used by workers with these viruses because no other method has been devised. In such a comprehensive book it is surprising to find no mention of this; it is worth mentioning because so many virus diseases of great economic importance, including practically all those of perennial plants, are transmissible only by insects or by grafting, so that nothing in the chapter on 'Quantitative Methods of Assaying for Viruses' is applicable to them. A. F. POSNETTE

Manual of Rice Diseases. By G. Watts Padwick. Pp. viii + 198. The Commonwealth Mycological Institute, Kew, Surrey, England. 1950. Price 30*s.* or $4.50.

Plant pathologists will receive with interest the *Manual of Rice Diseases* by G. Watts Padwick. The attempt of the author to bring together all the information available on diseases of rice has no doubt been very successful. The *Manual* has been divided into three parts; part I consisting of five chapters deals with the diseases of the foliage, diseases of the stem and leaf-sheath, seedling blights and foot-rot, diseases of the grain and inflorescence as well as those caused by nematodes. Part II consisting of three chapters deals with non-parasitic diseases caused by unfavourable soil conditions, deficiency and virus diseases. In part III a check-list of the fungi recorded on rice plant is provided.

The most important diseases on rice such as blast, brown-spot, stem-rot, foot-rot and Ufra have been dealt with at considerable length. Symptoms and the history of the diseases, as well as the control measures, have been thoroughly discussed. Along with each disease a bibliography is provided which facilitates reference to the original work by a researcher. The typical symptoms of stem-rot as described by Butler have been recorded, but in a rice field the haploid plants which are commonly found produce large numbers of tillers and remain sterile. Butler was obviously misled and considered this condition to have been brought about by the stem-rot fungus. It would have been useful if the author had cleared the position. The illustrations have been carefully drawn in the usual painstaking way in which Dr Padwick has so admirably put together all the information available on the diseases of the crop. The *Manual* is based on the author's experience in the rice-growing tracts of India as also during his office as Imperial Mycologist in India.

Ditylenchus angustus, the organism responsible for Ufra disease has been referred to as *Anguillulina angusta* in the preface. Also the virus diseases have been included under the chapter dealing with non-parasitic diseases.

The *Manual* will be of great value to a student of rice diseases, as it provides information on every desirable point. R. S. VASUDEVA

A Textbook of Biochemistry. By Philip H. Mitchell. Pp. xvii + 695. New York, Toronto, London: McGraw-Hill Book Co., Inc. 2nd edn., 1950. 51*s.*

This is not a text-book of biochemistry. The author's *Textbook of General Physiology* is described by the publishers as placing 'unusual emphasis' on human physiology. The work under review places an all too usual emphasis on vertebrate zoochemistry with particular reference to man. This does not detract from the usefulness of the book in these fields but the provision of a short chapter (18 pages) on photosynthesis, mention of a number of plant polysaccharides in the chapter on carbohydrates, and the inclusion of a number of fungal products in the chapter on chemotherapy is not enough to justify the title. The opportunity to redress the balance in the three-page 'Introduction to Biochemical Literature' has not been

taken. It would assist the student of biochemistry if his attention were directed to the existence of Stephenson's *Bacterial Metabolism*, Needham's *Biochemistry and Morphogenesis*, and Baldwin's *Introduction to Comparative Biochemistry* all of which deal with fields not touched on in the present work.

The first third of the book covers the chemistry of the carbohydrates, fats, proteins, and vitamins, the remainder being about equally divided between the biochemistry of the vertebrate organism as a whole and the biochemistry of the cell. The emphasis on intermediary metabolism in the cell is refreshing and the information is well presented. Great emphasis is laid on nutrition—the chapter on vitamins alone accounts for 10% of the text. Each chapter is supplemented with a bibliography of books, reviews, and selected papers. The reviewer found it irritating that no distinction was made in the text between references to be found in the bibliography and those which were not included. The book would be improved by more adequate cross-references. Thus it is implied on p. 108 that little is known of the essential amino-acid requirements of man, while on p. 482 a table is given of tentative values for these requirements. It is inevitable that a reviewer will find material omitted that he thinks necessary and material included that he thinks unnecessary. Nevertheless, it seems unjust that Astbury's original suggested structure for α-keratin should be illustrated and criticized although it was withdrawn by its author in 1941 and replaced by a more satisfactory formulation.

M. V. TRACEY

Le Parasitisme et la Symbiose. Par M. CAULLERY. Pp. 358+80 text-figures. Doin, Paris: Encyclopédie Scientifique. 2nd ed., 1948.

This book is the second edition of a work which first appeared thirty years ago. Its purpose is to discuss parasitism and closely related phenomena from the general biological point of view. It is divided into three parts, the first dealing with commensalism and inquilinism using both marine and terrestrial examples to illustrate the kind of adaptations, both anatomical and physiological, that occur as a result of associations of this kind, and their evolutionary implications. The second part is devoted to what is normally regarded as parasitism proper and includes such subjects as morphological and reproductive adaptations to the parasitic habit; temporary parasitism; migratory parasites; specificity and the reciprocal reactions of host and parasite. The final part of the book deals with symbiosis (the term being used in the more restricted sense) both among animals and plants and the final chapter is a discussion on the question of whether symbiosis is a primitive character of the cell.

The bibliography, which the author admits is not complete, contains some 582 references. It is somewhat irritating, however, to find for example on p. 288 a footnote making special mention of recent work without giving the reference to the worker's publications. There also seems no reason why references 189 and 190 should be sandwiched between references 237 and 238.

Nevertheless, the whole work is a straightforward account using a wide range of examples which enable the author logically to develop the subject. The discussion, which forms a considerable part of each chapter, prevents the work from being a mere catalogue and it should form a valuable addition to the literature on general biology.

L. R. JOHNSON

Symposia of the Society for Experimental Biology. Number IV. Physiological Mechanisms in Animal Behaviour. Pp. vii+482. Cambridge University Press. 1950. 35*s*.

This book is the record of a highly successful symposium held at Cambridge in 1949. It is a record of lasting value; for twenty-two contributors from many parts of the world presented critical accounts of those aspects of animal behaviour of which they had first-hand knowledge.

The range of topics was so wide and so well co-ordinated that the whole affords a more vivid picture of the present state of knowledge than could have been provided by a single author no matter how well informed.

The objective was to try and bridge the gap between our concepts of overt behaviour and the underlying neural mechanisms. The survey opens with an account of the range and capabilities of some of the sense organs. R. J. Pumphrey develops the thesis that hearing, though largely neglected, is perhaps the most important of the senses in animal behaviour. Katharine Tansley reviews the visual powers of vertebrates. Among the sense organs that are most needed for the proper co-ordination of the movements of the body are the proprioceptors (H. W. Lissmann); and for the maintenance of equilibrium in vertebrates the semicircular canals and the otolith organs, which have been a subject of renewed experiment in recent years, take a prominent part (O. Lowenstein).

Turning to the central and peripheral control of behaviour patterns E. D. Adrian uses the olfactory lobe of the rabbit to develop his conceptions of the way in which peripheral stimuli generate particular patterns of activity in the central nervous system. Paul Weiss describes again his experiments on the reinnervation of transposed and inverted limbs in Amphibia. The innervation follows a set anatomical pattern so that the resulting function in distorted limbs bears no relation to the needs of the animal. In the locomotion of vertebrates J. Gray can find no evidence of any central pattern of nervous activity; the moving limbs themselves appear to furnish the stimuli for each succeeding movement. But in the spontaneous cycles of activity seen in polychaete worms, behaviour is controlled by an internal 'pacemaker' within the nervous system (G. P. Wells); and E. von Holst (in German) uses two special responses in fishes (swimming on the back when illuminated from below; rotation around the long axis of the body when threatened by a rival) to illustrate the influence of central stimulation on equilibrium. Elaborate behaviour patterns in Coelenterates are mediated by an apparently simple 'nerve net' (C. F. A. Pantin). The starfish is sometimes a centrally motivated animal, sometimes it consists of a series of segments each with its hundreds of feet under reflex control (J. E. Smith).

The simplest level at which to study the behaviour of intact organisms is in the mechanisms of orientation. The lively account of this subject by O. Köhler shows that in Germany Kühn's useful scheme is still regarded as sacrosanct and that the merit of more recent developments is not yet appreciated. The longest and most entertaining paper is that in which K. Z. Lorenz describes in racy style his understanding of instinctive or innate behaviour patterns. By speaking of 'action-specific nervous energy' being stored up and flowing into specified channels when released by a specific stimulus, he is able to give a consistent description of many remarkable acts of behaviour in birds and other animals. N. Tinbergen elaborates this system further and postulates a hierarchy of nerve centres controlling instinctive behaviour. P. H. T. Hartley describes an experimental analysis of the recognition of predators by birds. G. P. Baerends considers the mechanisms by which instinctive reactions are released and E. A. Armstrong the peculiar 'displacement activities' which result in the performance of instinctive acts in inappropriate situations. There is a very wide gap between the conceptions of these authors and the ideas of the neurophysiologists. When they use neurophysiological terms it is well to realize that they do so in a figurative sense and that the 'centres' and 'energy' of which they speak have as yet no physical counterparts.

In the final section on learning the concepts and mechanisms of the process are reviewed by W. H. Thorpe and J. Konorski. E. B. Boycott and J. Z. Young describe the experimental study of learning in the octopus and squid and the effects on learning of operative injuries to the brain. And in the final paper K. S. Lashley describes the results of his thirty years' search for the 'memory trace' in the brain of rats and monkeys. It is a humbling contribution for it shows how far we are from understanding the workings of the brain once we look inside the animal.

V. B. WIGGLESWORTH

Analytical Biology. By G. SOMMERHOFF. Oxford University Press (London: Geoffrey Cumberlege). Pp. viii + 208. 1st ed. 1950. 17*s*. 6*d*.

This book sets out to discuss what is meant by biological organization, as it appears to exist, for example, in the organization and purposive behaviour of a colony of ants. The author errs when he writes: 'Only the physico-mathematical language offers the highest attainable level of both formal and semantic exactitude and definiteness.' For mathematics is no more than a language. The English, French, and mathematical versions of a statement can be equally precise; though the mathematical one ought to be the most concise. Mathematics loses its only advantage if, as here, an author misuses it to become the more prolix. For instance, on p. 120 he proposes that $P_{t_1} = Q_{t_1}$ and he concludes that

$$\frac{\partial P_{t_1}}{\partial Q_{t_0}} = \frac{\partial Q_{t_1}}{\partial Q_{t_0}};$$

that is to say, a quantity behaves in the same fashion whichever of two alternative symbols P_{t_1} or Q_{t_1} denotes it. Sommerhoff employs four intermediate equations and half a page of argument to arrive at this conclusion: whereas with equal precision and much greater clarity Shakespeare said in a line and a half 'that which we call a rose By any other name would smell as sweet'.

The emptiness of the mathematical thought, the over-elaborate notation, the profusion of mathematical prolixities and errors, which pervade this book, make the mathematical reader suspect that the polysyllabic biological side is equally useless and inflated.

J. M. HAMMERSLEY

THE ANNALS OF APPLIED BIOLOGY

The Annals of Applied Biology is conducted by the Association of Applied Biologists and is published by the Cambridge University Press. The Association exists to further the study of all aspects of Biology and to correlate pure science with practice. Meetings are held about six times a year for informal discussion and the reading of papers. Papers from non-members must be communicated by a Member.

The annual subscription to the Association, which includes a copy of the *Annals*, is 25*s*. (entrance fee 12*s*. 6*d*.), becomes due on 1 Jan. of each year and should be paid to the Treasurer.

The price of the *Annals* to non-members of the Association is £2. 10*s*. 0*d*. per volume or $8.75 in U.S.A. Such subscriptions (payable in advance) and all communications respecting the publication should be sent to The Cambridge University Press, 200 Euston Road, London, N.W. 1, or in U.S.A. to the American Branch of the Press at 51 Madison Avenue, New York, 10.

Claims for missing numbers should be made within the month following that of regular publication.

Quotations can be given for binding cases and for binding subscribers' sets; (suspended) also for bound copies of back volumes.

Vols. I to V of the *Annals* will be supplied at the following reduced rates:—To Members of the Association, single volumes 10*s*., single parts 5*s*.; double numbers, 8*s*. 6*d*. Complete sets I–V £1. 10*s*. 0*d*. To non-members of the Association, single volumes 15*s*., single parts 7*s*. 6*d*.; double numbers 10*s*. Complete sets I–V £2. 5*s*. 0*d*.

Notice to Contributors

MANUSCRIPTS FOR PUBLICATION.

Contributors should address all material to Mr R. W. Marsh, Research Station, Long Ashton, Bristol. Manuscripts submitted for publication must be type-written, double spaced with ample margins, on one side only of white opaque paper approximately 13 × 8 in. It is understood that they are not offered to any other Journal for prior or simultaneous publication. Preference will be given to Members' papers: papers from non-members must be communicated by a Member for consideration by the Editors.

Owing to the demand for space and the high cost of printing, authors must drastically condense their manuscripts, in the general interest of all contributors. Numerical results should be presented either as tables or as diagrams, but not both: only such tables and illustrations as are essential can be published. Papers must start with a summary and conform to the usages of the *Annals* in all typographical matters. Contributors will be responsible for any excess over the usual charges allowed for corrections. Papers over 8000 words can be published only in exceptional circumstances. Quantitative biological data too extensive for complete publication should be deposited with the Librarian, British Museum (Natural History), Cromwell Road, London, S.W. 7.

REFERENCES.

References must be listed at the end of the article according to the "Harvard System" as follows: name and initial(s) of author; year of publication in brackets, further distinguished by the addition of small letters *a*, *b*, *c* to the date where more than one paper published by the same author(s) in the same year is cited; exact title of paper; contracted title of periodical as given in *World List of Scientific Periodicals*; volume number in arabic figures; beginning page number of article. In the text references should be denoted by giving the name of the author(s) with the date of publication in brackets, e.g. (Brown, 1937), (Brown, 1937*a*; Jones & Smith, 1942*a*, *b*). In the References *all* names should be given in full but, where more than *two* collaborating authors are quoted in the text, the names are printed in full only at the first citation; after that the first name is followed by *et al*.

ILLUSTRATIONS.

Text illustrations accompanying the papers should be drawn on smooth white Bristol board in Indian ink about twice the size of the finished block. Shading must be indicated by lines or dots. Graphs should be drawn boldly in Indian ink on white Bristol board or on blue-lined white graph paper. All lettering of illustrations or graphs should be inserted clearly in pencil.

SEPARATES.

Fifty separates of each paper are provided free.

Printed in Great Britain at the University Press, Cambridge (Brooke Crutchley, University Printer)